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Indications and limitations for aged patients with chronic hepatitis C in pegylated interferon alfa-2b plus ribavirin combination therapy

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Background & Aims: This study investigated the efficacy and adverse effects of pegylated interferon (Peg-IFN) plus ribavirin therapy in aged patients with chronic hepatitis C (CH-C).

Methods: A total of 1040 naïve patients with CH-C (genotype 1, $n = 759$; genotype 2, $n = 281$), of whom 240 (23%) over 65 years old (y.o.), were treated with Peg-IFN alfa-2b plus ribavirin and assessed after being classified into five categories, according to age.

Results: The discontinuance rate was higher for patients over 70 y.o. (36%), the most common reason being anemia. In the presence of genotype 1, the SVR rate was similar (42–46%) among patients under 65 y.o. and declined (26–29%) among patients over 65 y.o. For patients over 65 y.o., being male (Odds ratio, OR, 3.5, $p = 0.035$) and EVR (OR, 83.3, $p < 0.001$) were significant factors for SVR, in multivariate analysis. The Peg-IFN dose was related to EVR, and when EVR was attained, 76–86% of patients over 65 y.o. achieved SVR. SVR was not achieved (0/35, 0/38, respectively) if a 1-log decrease and a 2-log decrease were not attained at week 4 and week 8, respectively. In the presence of genotype 2, the SVR rate was similar (70–71%) among patients under 70 y.o. and declined among patients over 70 y.o. (43%).

Conclusions: Aged patients up to 65 y.o. with genotype 1 and 70 y.o. with genotype 2 can be candidates for Peg-IFN plus ribavirin therapy. The response-guided therapy can be applied for aged patients with genotype 1.

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Introduction

Pegylated interferon (Peg-IFN) plus ribavirin combination therapy has led to a marked progress in the treatment of chronic hepatitis C (CH-C) [1–4]. However, in aged patients, problems remain with respect to its anti-viral effect and tolerability [5–9]. Recently, the addition of a protease inhibitor to Peg-IFN plus ribavirin combination therapy has been reported, on the one hand, to improve the anti-viral effect, and, on the other hand, to increase side effects, especially severe anemia [10–11].

Therefore, this new therapy does not solve the problems encountered when treating aged patients.

With aging, the progression of liver fibrosis and the occurrence of hepatocellular carcinoma (HCC) have been shown to be accelerated, especially in patients over 60 y.o. [12–14]. In general, the anti-viral therapy can lead to an improvement in liver fibrosis and thus diminish the risk of HCC and ameliorate the prognosis in patients with CH-C [15–21]. Among aged patients, those results are mainly achievable upon eradication of the hepatitis C virus (HCV) [18,21]. Accordingly, the first goal of treatment of aged patients with a high-risk of HCC should be HCV elimination.

Thus, a treatment strategy, aiming at the improvement of the anti-viral efficacy in aged patients, should be established based on detailed large-scale studies.

Some points need to be further elucidated when using the Peg-IFN plus ribavirin combination therapy for the treatment of aged patients with CH-C: (i) the characteristics before treatment

Keywords: Pegylated interferon plus ribavirin therapy; Chronic hepatitis C; Aged patients.

Received 30 April 2010; received in revised form 28 July 2010; accepted 28 July 2010; available online 9 December 2010

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Abbreviations: HCV, hepatitis C virus; CH-C, chronic hepatitis C; HCC, hepatocellular carcinoma; Peg-IFN, pegylated interferon; SVR, sustained virologic response; RVR, rapid virologic response; EVR, early virologic response; LVR, late virologic response; NR, non-response; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Plt, platelet; G-CSF, granulocyte-macrophage colony stimulating factor.



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that would lead to the successful elimination of HCV, (ii) the prediction factors of treatment efficacy after the initiation of the therapy, and (iii) the utility of a response-guided therapy established in the treatment.

In the present study, using a large cohort, we aimed at clarifying these points taking into account the patients' age.

Patients and methods

Patients

This study was a retrospective, multicenter trial conducted by the Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 1040 naïve patients with CH-C were enrolled between December 2004 and June 2007. All patients were Japanese, infected with a viral load of more than 10^5 IU/ml, and treated with a combination of Peg-IFN alfa-2b plus ribavirin. Patients were excluded from the study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), coinfection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

Treatment

All patients received Peg-IFN alfa-2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough). Treatment duration was 48 weeks for patients with genotype 1 and 24 weeks for those with genotype 2. As a starting dose, Peg-IFN alfa-2b was given once weekly, at a dosage of 1.5 µg/kg, and ribavirin was given at a total dose of 600–1000 mg/day based on body weight (body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction and discontinuance

Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematologic adverse effects. The Peg-IFN alfa-2b dose was reduced to 50% of the assigned dose when the white blood cell (WBC) count was below $1500/\text{mm}^3$, the neutrophil count below $750/\text{mm}^3$ or the platelet (Plt) count below $8 \times 10^9/\text{mm}^3$, and was discontinued when the WBC count was below $1000/\text{mm}^3$, the neutrophil count below $500/\text{mm}^3$ or the Plt count below $5 \times 10^9/\text{mm}^3$. Ribavirin was also reduced from 1000 to 600 mg, 800 to 600 mg, or 600 to 400 mg when the hemoglobin (Hb) was below 10 g/dl, and was discontinued when the Hb was below 8.5 g/dl. Peg-IFN alfa-2b and ribavirin had to be both discontinued if there was a need to discontinue either of them. No ferric medicine or hematopoietic growth factors, such as epoetin alpha, or granulocyte-macrophage colony stimulating factor (G-CSF), were administered.

Virologic assessment and definition of virologic response

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml; Roche Diagnostics, Branchburg, NJ) and qualitatively analyzed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/ml; Roche Diagnostics). The rapid virologic response (RVR) was defined as undetectable serum HCV RNA at week 4; the early virologic response (EVR) as undetectable serum HCV RNA at week 12; and the late virologic response (LVR) as detectable serum HCV RNA at week 12 and undetectable serum HCV RNA at week 24. Moreover, the sustained virologic response (SVR) was defined as undetectable serum HCV RNA, 24 weeks after treatment.

According to the protocol, genotype 1 patients, with less than a 2-log decrease in HCV RNA level at week 12 compared to the baseline, or with detectable serum HCV RNA at week 24, had to stop the treatment and were regarded as non-response (NR). Treatment discontinuance was evaluated except for those patients who had discontinued the treatment at up to 24 weeks, due to absence of response. Anti-viral efficacy was evaluated, for all study patients, using the intention-to-treat analysis (ITT analysis) and the per protocol analysis (PP analysis) for patients without treatment discontinuation due to side effects, and was assessed considering the definition of EVR or LVR for genotype 1, and RVR or non-RVR for genotype 2, as previously reported [1].

Assessment of drug exposure

The amounts of Peg-IFN alfa-2b and ribavirin, taken by each patient during the full treatment period, were evaluated by reviewing the medical records. The mean doses of Peg-IFN alfa-2b and ribavirin were calculated individually as averages, on the basis of the body weight at baseline: Peg-IFN alfa-2b expressed as µg/kg/week, ribavirin expressed as mg/kg/day.

Statistical analysis

Patients' baseline data are expressed as means ± SD or median values. To analyze the difference between baseline data, ANOVA or Mantel-Haenszel Chi-square test were performed. Factors associated with the viral response were assessed by univariate analysis using the Mann-Whitney *U* test or Chi-square test and multivariate analysis using logistic regression analysis. A two-tailed *p* value <0.05 was considered significant. The analysis was conducted with SPSS version 15.0J (SPSS Inc., Chicago, IL).

Results

Patient's profile

Baseline characteristics of the patients categorized by age are shown in Table 1.

Genotype 1 patients (*n* = 759) were distributed into five categories: 266 patients were under 55 y.o. (group 1A), 159 were 55–59 y.o. (group 1B), 149 were 60–64 y.o. (group 1C), 134 were 65–69 y.o. (group 1D), and 51 were 70 y.o. or older (group 1E). With advancing age, the male-to-female ratio and peripheral blood cell count (WBC, neutrophil count, Red blood cell (RBC), Hb, Plt) decreased significantly. Patients with a progression of liver fibrosis (METAVIR fibrosis score 3 or 4) significantly increased with age (Table 1A).

Genotype 2 patients (*n* = 281) were also distributed into five categories: 145 patients were under 55 y.o. (group 2A), 43 were 55–59 y.o. (group 2B), 38 were 60–64 y.o. (group 2C), 41 were 65–69 y.o. (group 2D), and 14 were 70 y.o. or older (group 2E). As observed in genotype 1 patients, the peripheral blood cell count decreased and the ratio of advanced fibrosis (score 3–4) increased significantly with age (Table 1B). For both genotypes, the initial doses of Peg-IFN in patients over 70 y.o. were lower than in those under 70 y.o., this was not the case for the ribavirin doses.

Dose reduction and discontinuance for adverse event

The overall discontinuance rate of treatment was 15% (140/919); 18% (112/639) for genotype 1 and 10% (28/280) for genotype 2, respectively. Table 2 shows the reason for and the rate of treatment discontinuance according to age. The discontinuance rate increased with age, being 10% (36/363) for patients under 55 y.o., 15% (27/182) for patients with 55–59 y.o., 17% (28/169) for patients with 60–64 y.o., 19% (28/147) for patients with 65–70 y.o., and significantly higher, 36%, (21/58) for patients over 70 y.o. The discontinuance of treatment due to hemolytic anemia was significantly higher for patients over 70 y.o. as compared to those under 70 y.o. (<70 y.o., 1% (9/861) vs. ≥70 y.o., 16% (9/58), *p* <0.0001).

The rate without dose reduction of both drugs decreased with age (<55 y.o., 41% (171/411); 55–59 y.o., 20% (40/202); 60–64 y.o., 26% (48/187); 65–69 y.o., 23% (41/175); ≥70 y.o., 18% (12/65)). In the presence of genotype 1, the mean dose of Peg-IFN

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Table 1. Baseline characteristics of patients.

Patients with genotype 1							
Factor	<55 y.o.	55 - 59 y.o.	60 - 64 y.o.	65 - 69 y.o.	≥70 y.o.	p value	
Number	266	159	149	134	51		
Age (y.o.)	44.4 ± 8.1	56.9 ± 1.4	62.0 ± 1.4	66.8 ± 1.4	71.4 ± 1.7	<0.001	
Sex: male / female	160 / 106	64 / 95	57 / 92	54 / 80	23 / 28	<0.001	
Body weight (kg)	64.6 ± 11.7	58.3 ± 9.4	58.1 ± 9.6	56.3 ± 9.3	56.3 ± 9.2	<0.001	
White blood cells (/mm ³)	5608 ± 1668	4901 ± 1664	4888 ± 1488	5113 ± 1426	4883 ± 1511	<0.001	
Neutrophils (/mm ³)	2923 ± 1214	2425 ± 1031	2559 ± 1155	2535 ± 1017	2599 ± 1149	<0.001	
Red blood cells (×10 ⁶ /mm ³)	454 ± 47	432 ± 38	427 ± 40	424 ± 37	424 ± 46	<0.001	
Hemoglobin (g/dl)	14.4 ± 1.5	13.8 ± 1.2	13.7 ± 1.3	13.6 ± 1.2	13.7 ± 1.4	<0.001	
Platelets (×10 ⁴ /mm ³)	18.6 ± 6.2	16.3 ± 5.7	15.4 ± 5.3	15.1 ± 5.0	14.4 ± 4.2	<0.001	
AST (IU/L)	62 ± 50	62 ± 45	64 ± 46	72 ± 45	64 ± 40	0.295	
ALT (IU/L)	79 ± 68	76 ± 64	73 ± 63	77 ± 58	65 ± 41	0.657	
Serum HCV RNA (KIU/ml)*	1800	1600	1700	1700	1700	0.691	
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	177 / 19	99 / 20	90 / 19	76 / 28	21 / 9	0.001
		Activity, 0 - 1 / 2 - 3	117 / 79	63 / 56	59 / 50	47 / 57	13 / 16
Peg-IFN dose (μg/kg/week)‡	1.47 ± 0.14	1.47 ± 0.16	1.46 ± 0.18	1.44 ± 0.18	1.36 ± 0.24	<0.001	
Ribavirin dose (mg/kg/day)¶	11.5 ± 1.1	11.5 ± 1.4	11.5 ± 1.4	11.5 ± 1.7	11.2 ± 2.2	0.65	

Patients with genotype 2							
Factor	<55 y.o.	55 - 59 y.o.	60 - 64 y.o.	65 - 69 y.o.	≥70 y.o.	p value	
Number	145	43	38	41	14		
Age (y.o.)	40.9 ± 8.9	56.7 ± 1.3	62.3 ± 1.4	66.7 ± 1.5	71.8 ± 1.8	<0.001	
Sex: male / female	78 / 67	17 / 26	17 / 21	18 / 23	6 / 8	0.441	
Body weight (kg)	63.4 ± 12.0	59.5 ± 11.5	58.6 ± 11.7	58.5 ± 9.8	55.9 ± 6.8	0.783	
White blood cells (/mm ³)	6011 ± 1965	4874 ± 1346	4982 ± 1210	5079 ± 1877	4414 ± 871	<0.001	
Neutrophils (/mm ³)	3214 ± 1511	2468 ± 971	2576 ± 950	2492 ± 1119	2521 ± 683	0.001	
Red blood cells (×10 ⁶ /mm ³)	454 ± 48	430 ± 42	432 ± 50	430 ± 43	408 ± 48	<0.001	
Hemoglobin (g/dl)	14.3 ± 1.6	13.5 ± 1.3	13.9 ± 1.4	13.9 ± 1.3	13.3 ± 1.2	0.001	
Platelets (×10 ⁴ /mm ³)	21.3 ± 5.4	18.3 ± 6.1	17.0 ± 5.2	15.8 ± 5.4	13.9 ± 4.7	<0.001	
AST (IU/L)	53 ± 59	57 ± 45	55 ± 38	83 ± 48	68 ± 29	0.029	
ALT (IU/L)	65 ± 59	73 ± 70	68 ± 62	105 ± 62	78 ± 43	0.008	
Serum HCV RNA (KIU/ml)*	1700	1100	900	1100	500	0.008	
Histology (METAVIR)‡	Fibrosis, 0 - 2 / 3 - 4	102 / 0	25 / 3	29 / 2	21 / 9	7 / 1	<0.001
		Activity, 0 - 1 / 2 - 3	68 / 34	18 / 10	18 / 13	9 / 21	5 / 3
Peg-IFN dose (μg/kg/week)‡	1.48 ± 0.16	1.48 ± 0.14	1.45 ± 0.18	1.46 ± 0.15	1.28 ± 0.26	0.001	
Ribavirin dose (mg/kg/day)¶	11.5 ± 1.1	11.4 ± 1.2	11.5 ± 1.4	11.3 ± 1.6	11.0 ± 1.4	0.55	

*, Data shown are median values.

†, 201 Missing.

‡, 82 Missing.

¶, Initial doses.

during the whole treatment period was lower (1.1 ± 0.3 μg/kg/week) for patients over 70 y.o. than for those under 70 y.o. (1.3 ± 0.3 μg/kg/week) and that of ribavirin decreased with age (<55 y.o., 10.3 ± 1.9 mg/kg/day; 55–59 y.o., 9.8 ± 1.9 mg/kg/day; 60–64 y.o., 9.3 ± 2.3 mg/kg/day; 65–69 y.o., 9.2 ± 2.3 mg/kg/day; ≥70 y.o., 8.5 ± 2.5 mg/kg/day). The same tendency was observed with genotype 2.

Sustained virologic response

In genotype 1 patients, the overall SVR rate was 40% (305/759), being 46% (123/266) for group 1A, 44% (70/159) for group 1B, 42% (62/149) for group 1C, 26% (35/134) for group 1D, and 29% (15/51) for group 1E, following ITT analysis. The same tendency was observed using the PP analysis ($n = 647$). The SVR rates for patients over 65 y.o. were significantly lower than those for patients under 65 y.o. (ITT analysis: ≥65 y.o., 27% vs. <65 y.o.,

44%, $p < 0.0001$; PP analysis: ≥65 y.o., 31% vs. <65 y.o., 50%, $p < 0.0001$) (Fig. 1A). Among genotype 1 patients over 65 y.o., the SVR rate was significantly lower for female patients than for male patients (ITT analysis: male, 40% (31/77) vs. female, 18% (19/108), $p < 0.001$; PP analysis: male, 49% (27/55) vs. female, 20% (18/90), $p < 0.001$).

Moreover, for genotype 2 patients, the overall SVR rate was 78% (220/281), being 88% (128/145) for group 2A, 70% (30/43) for group 2B, 71% (27/38) for group 2C, 71% (29/41) for group 2D, and 43% (6/14) for group 2E, following ITT analysis. The same tendency was observed with the PP analysis ($n = 253$). The SVR rates for patients over 70 y.o. were significantly lower than those for patients under 70 y.o. (ITT analysis: ≥70 y.o., 43% vs. <70 y.o., 80%, $p < 0.0001$; PP analysis: ≥70 y.o., 56% vs. <70 y.o., 85%, $p < 0.05$) (Fig. 1B). Among patients over 70 y.o. with genotype 2, the difference according to gender was not clear because of the small sample.

Table 2. Reasons for treatment discontinuation.

Factor	<55 y.o. (n = 363)	55 - 59 y.o. (n = 182)	60 - 64 y.o. (n = 169)	65 - 69 y.o. (n = 147)	≥70 y.o. (n = 58)	Total (n = 919)
Neutropenia	2	3	0	0	0	5
Thrombopenia	1	0	1	1	0	3
Anemia	0	4	3	2	9	18
Fatigue	1	1	3	3	1	9
Gastrointestinal disorder	2	1	0	0	1	4
Cough, Dyspnea	1	0	3	0	0	4
Vertigo	1	0	0	0	3	4
Psychosis (depression)	7 (3)	7 (3)	4 (4)	3 (3)	2 (2)	23
Rash	5	2	5	7	1	20
Thyroid dysfunction	2	0	2	0	0	4
Fundal hemorrhage	0	2	0	2	0	4
Drug-induced hepatitis	3	1	0	0	0	4
Interstitial pneumonia	0	1	0	1	1	3
Cerebral hemorrhage, infarction	2	0	0	1	0	3
Others	9	5	7	8	3	32
Total	36 (10%)	27 (15%)	28 (17%)	28 (19%)	21 (36%)	140 (15%)

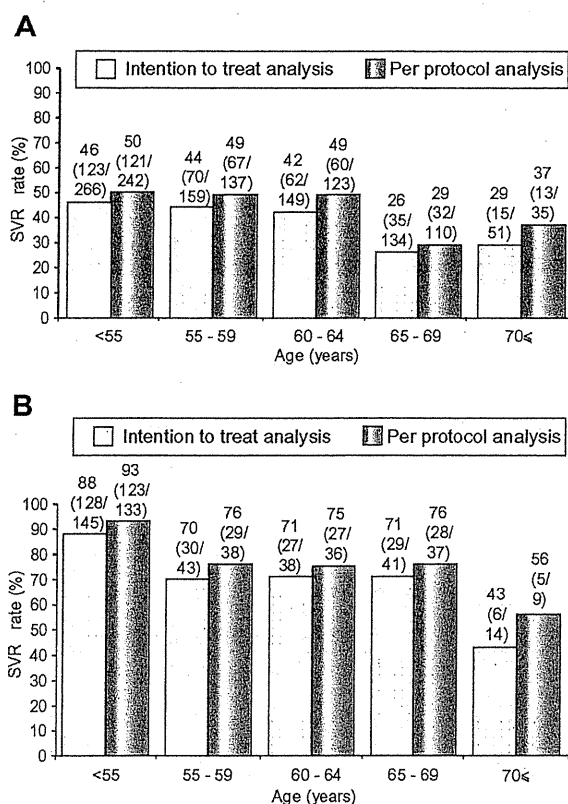


Fig. 1. SVR rate according to age. (A) Genotype 1. (B) Genotype 2.

Timing of HCV RNA negativation for genotype 1, according to age

Treatment responses distributing EVR, LVR, and NR according to age are shown in Fig. 2. The rates of NR were similar in patient groups under 65 y.o. (30–36%), but increased in almost half of

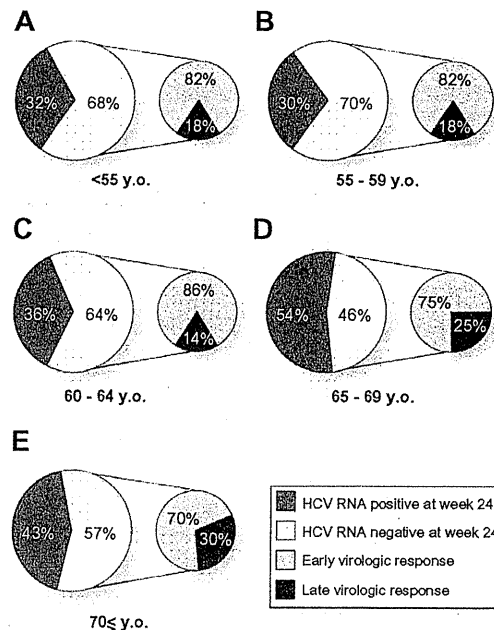


Fig. 2. Antiviral effect during treatment according to age. (A) <55 y.o. (B) 55–59 y.o. (C) 60–64 y.o. (D) 65–69 y.o. (E) ≥70 y.o.

the patients over 65 y.o. ($p < 0.0001$). Moreover, among the virologic responders, the proportion of LVR tended to increase in patients over 65 y.o. (25–30%) compared to patients under 65 y.o. (14–18%) ($p = 0.06$).

SVR rate according to the timing of HCV RNA negativation

SVR rates according to EVR or LVR in genotype 1, and RVR or non-RVR in genotype 2 are summarized in Table 3. Genotype 1 patients with EVR achieved high SVR rates regardless of age; in particular, if EVR had been attained, 76% of patients with 65–69

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Table 3. SVR rate according to genotype and viral response in patients responding to PEG-IFN plus ribavirin combination therapy.

Factor	<55 y.o.	55 - 59 y.o.	60 - 64 y.o.	65 - 69 y.o.	≥70 y.o.
Genotype 1					
with EVR, % (n)	85 (114/134)	79 (62/79)	81 (55/68)	76 (29/38)	86 (12/14)
with LVR, % (n)	23 (7/30)	29 (5/17)	46 (5/11)	23 (3/13)	17 (1/6)
Genotype 2					
with RVR, % (n)	93 (57/61)	82 (14/17)	85 (17/20)	92 (11/12)	100 (4/4)
without RVR*, % (n)	96 (22/23)	60 (6/10)	57 (4/7)	50 (4/8)	0 (0/3)

RVR, rapid virologic response.

EVR, early virologic response.

LVR, late virologic response.

*, Serum HCV RNA was detectable at week 4, but undetectable at week 24.

Table 4. Multivariate analysis for the factors associated with SVR among all patients.

Factor	Category	Odds ratio	95% CI	<i>p</i>
Age (y.o.)	<65 / ≥65	0.485	0.295 - 0.799	0.005
Sex	male / female	0.524	0.353 - 0.777	0.001
Platelets ($\times 10^4/\text{mm}^3$)	<12 / ≥12	1.780	1.039 - 3.049	0.040
Serum HCV RNA (KIU/ml)	<2000 / ≥2000	0.599	0.401 - 0.896	0.010
Histology (METAVIR): Fibrosis	0 - 2 / 3 - 4	0.599	0.333 - 1.076	0.090

y.o. and 86% of patients over 70 y.o. achieved SVR, and these SVR rates compared favorably with those of younger patients. On the other hand, the SVR rates for patients with LVR ranged from 17% to 46%, which were lower than those for EVR patients in each age group, and no significant differences of SVR rates were found among LVR patients by age.

With genotype 2, patients with RVR achieved high SVR rates ranging from 82% to 100% regardless of age. Even for patients without RVR, 96% of those under 55 y.o. attained SVR, a rate that was significantly higher than that for patients over 55 y.o. (50%, 14/28) ($p < 0.001$).

Factors associated with SVR for genotype 1

The factors associated with SVR were assessed for the variables shown in Table 1. The factors selected as significant by the univariate analysis: age, gender, WBC, neutrophils, RBC, Hb, Plt, aspartate aminotransferase, serum HCV RNA level, the degree of liver fibrosis, and the initial dose of Peg-IFN, were evaluated by multivariate logistic regression analysis. The factor of age over 65 y.o. was the independent factor for SVR ($p = 0.005$), apart from the gender ($p = 0.001$), Plt value ($p < 0.05$), and serum HCV RNA level ($p = 0.01$) (Table 4).

Factors associated with EVR and SVR for patients over 65 y.o. with genotype 1

The results of univariate analysis for EVR among patients over 65 y.o. are shown in Table 5A. Gender, Plt value, and mean dose of Peg-IFN during the first 12 weeks were factors significantly associated with EVR. In multivariate analysis, the mean dose of Peg-IFN during the first 12 weeks was the independent factor for EVR ($p = 0.03$), apart from gender ($p = 0.002$) (Table 5B). The EVR rates were 41% (41/101) in patients who received ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$ on average during the first 12 weeks, and declined to 36% (8/22) in patients given 0.9–1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN, and

to 14% (3/22) in patients administered with < 0.9 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN.

The baseline and on-treatment factors, which are correlated with the SVR among the patients over 65 y.o., were assessed by univariate and multivariate analyses. Univariate analysis showed that factors significantly associated with SVR were gender and virologic response (Table 6A), and they were also selected as significant independent factors in multivariate analysis ($p = 0.035$, $p < 0.001$) (Table 6B).

Negative prediction of SVR for patients over 65 y.o. with genotype 1

We tried positive and negative predictions of SVR for aged patients, focusing on the decrease of HCV RNA at treatment week 4 and 8. The SVR rate was 47% (29/62) for patients with more than a 1-log decrease in HCV RNA level at week 4, while no patients with less than a 1-log decrease at week 4 attained SVR (0/35) ($p < 0.0001$). Similarly, 55% (35/64) of patients with more than a 2-log decrease at week 8 attained SVR, whereas no patients with less than a 2-log decrease at week 8 attained SVR (0/38) ($p < 0.0001$).

Discussion

Peg-IFN plus ribavirin combination therapy can improve anti-viral efficacy and is presently recommended as first-line therapy [1–4]. However, with respect to aged patients with CH-C, there have been only a few small-scale cohort studies which reported poor anti-viral effect and poor tolerability in comparison with non-aged patients [5–9]. The problem in the treatment of aged patients with CH-C is most serious in Japan, because HCV carriers in Japan are 10–20 years older than those in the United States and European countries [22]. Therefore, in the present study, we examined the efficacy and prevalence of side effects with a focus on patient's age using a large-scale cohort.

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Table 5. Factors associated with EVR among patients over 65 y.o.

Univariate analysis				
Factor		EVR	Non-EVR	p value
Number		52	93	
Age (y.o.)		67.9 ± 2.3	67.8 ± 2.5	0.66
Sex: male / female		28 / 24	27 / 66	0.003
White blood cells (/mm ³)		5063 ± 1474	5001 ± 1422	0.76
Neutrophils (/mm ³)		2566 ± 1110	2551 ± 1071	0.87
Red blood cells (×10 ⁴ /mm ³)		426 ± 36	421 ± 38	0.64
Hemoglobin (g/dl)		13.7 ± 1.2	13.5 ± 1.2	0.21
Platelets (×10 ⁴ /mm ³)		16.5 ± 5.5	14.0 ± 4.6	0.009
AST (IU/L)		70 ± 51	70 ± 40	0.49
ALT (IU/L)		76 ± 58	70 ± 41	0.80
Serum HCV RNA (KIU/ml)*		1700	1900	0.62
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	25 / 10	47 / 20	0.54
	Activity, 0 - 1 / 2 - 3	16 / 19	29 / 37	0.52
Peg-IFN dose (µg/kg/week)‡		1.35 ± 0.24	1.25 ± 0.31	0.03
Ribavirin dose (mg/kg/day)‡		10.0 ± 2.2	9.6 ± 2.3	0.40
Multivariate analysis				
Factor	Category	Odds ratio	95% CI	p value
Sex	male / female	0.309	0.149 - 0.644	0.002
Platelets (×10 ⁴ /mm ³)	<12 / ≥12	-	-	N.S.
Peg-IFN dose (µg/kg/week)‡	<1.2 / ≥1.2	2.481	1.079 - 5.705	0.03

*, Data shown are median values.

†, 43 Missing.

‡, Mean doses during 0 to 12 weeks.

N.S., not statistically significant.

Table 6. Factors associated with SVR among patients over 65 y.o.

Univariate analysis				
Factor		SVR	Non-SVR	p value
Number		45	100	
Age (y.o.)		68.0 ± 2.4	67.7 ± 2.5	0.45
Sex: male / female		27 / 18	28 / 72	<0.001
White blood cells (/mm ³)		5006 ± 1516	5030 ± 1409	0.81
Neutrophils (/mm ³)		2575 ± 1130	2548 ± 1063	0.96
Red blood cells (×10 ⁴ /mm ³)		427 ± 40	421 ± 36	0.53
Hemoglobin (g/dl)		13.8 ± 1.3	13.5 ± 1.2	0.14
Platelets (×10 ⁴ /mm ³)		16.1 ± 5.6	14.3 ± 4.7	0.09
AST (IU/L)		71 ± 54	69 ± 40	0.47
ALT (IU/L)		76 ± 56	70 ± 43	0.77
Serum HCV RNA (KIU/ml)*		1700	2000	0.51
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	21 / 8	51 / 22	1.00
	Activity, 0 - 1 / 2 - 3	14 / 15	31 / 41	0.66
Peg-IFN dose (µg/kg/week)‡		1.27 ± 0.28	1.23 ± 0.33	0.31
Ribavirin dose (mg/kg/day)‡		8.8 ± 2.1	9.1 ± 2.5	0.38
Virologic response: EVR / non-EVR		41 / 4	11 / 89	<0.001
Multivariate analysis				
Factor	Category	Odds ratio	95% CI	p value
Sex	male / female	0.283	0.088 - 0.914	0.035
Virologic response	EVR / non-EVR	0.012	0.004 - 0.043	<0.001

*, Data shown are median values.

†, 43 Missing.

‡, Mean doses during treatment.

Research Article

With respect to the side effects and discontinuance rate of treatment in aged patients with CH-C, treated with Peg-IFN plus ribavirin combination therapy, Reddy et al. reported that there was no difference related to the incidence and reason for side effects between non-aged and aged patients [6]. Another paper reported that the incidence of side effects was more frequent in aged patients [5]. In our study, not only the continuance rate without reduction of both drug decreased with age, but also the discontinuance rate of treatment increased with age, with a third of the patients over 70 y.o. discontinuing the treatment. The discrepancy, existing between our results and those reported in the former study cited above, is due to the difference in the number of aged patients enrolled; Reddy's study analyzed a small cohort including only a few cases of patients over 65 y.o. and classified all those over 50 y.o. as aged patients.

Discontinuance of treatment due to progression of anemia was significantly higher in patients over 70 y.o., accounting for 43% (9/21) of the discontinuance in this group. Although the ratio of advanced fibrosis (score 3–4) increased with age, the high discontinuance rate due to anemia among patients over 70 y.o. was similar regardless of the progression of fibrosis (F0–2: <70 y.o., 1% (6/559) vs. ≥70 y.o., 21% (6/28), $p < 0.0001$; F3–4: <70 y.o., 0% (0/83) vs. ≥70 y.o., 22% (2/9), $p < 0.0001$). It is possible that poor hematopoietic function and renal function led to the progression of anemia in aged patients. For patients who develop severe anemia, using epoetin alpha or taribavirin, which are ribavirin pro-drugs, has been shown to result in a lower incidence of anemia, although no significant increase of SVR has been reported so far, even with the addition of taribavirin to Peg-IFN [23–24].

With genotype 1 patients, the SVR rates were almost equal up to 65 y.o. (49–50%), but decreased to 31% (45/145) among the patients that were over 65 y.o., and even for those who completed the entire treatment schedule in this study. Since the degree of liver fibrosis and drug exposure have been shown to be associated with anti-viral efficacy, the progression of liver fibrosis or decrease of drug exposure with age could account for the reduction of SVR rate among the aged patients. However, the stratified analysis, according to the progression of liver fibrosis and drug exposure, revealed that older patients still yielded low a SVR rate (F0–2, Peg-IFN during the first 12 weeks ≥1.2 µg/kg/week: <65 y.o., 55% (143/261) vs. ≥65 y.o., 33% (15/46), $p < 0.0001$; F0–2, Peg-IFN during the first 12 weeks <1.2 µg/kg/week: <65 y.o., 43% (26/60) vs. ≥65 y.o., 23% (6/26), $p = 0.07$), which means that older patients would be difficult to treat. From our results showing a low SVR rate and a high discontinuance rate for patients over 65 y.o., the genotype 1 patients under 65 y.o. were those who benefited the most from Peg-IFN plus ribavirin combination therapy. The high prevalence of treatment failure (non-SVR) among the aged patients seems to be due to the high populations of NR and LVR (Fig. 2). A high population of LVR is considered to lead to a higher transient response rate among aged patients, since those over 65 y.o. with LVR showed a much higher relapse rate (79%, 15/19) than those with EVR (21%, 11/52) ($p < 0.0001$), as can be seen from Table 3.

In this study, multivariate analysis for SVR, in patients over 65 y.o., showed that the factors associated with SVR were EVR and gender. This indicates that better SVR can be expected even with older patients if EVR is attained and response-guided therapy guidelines can be useful for aged patients. A low SVR rate among aged female patients was as previously reported [7], although the

mechanism remains unclear. This finding suggests that female patients should be treated before 65 y.o.

The next question is how aged patients should be treated in order to attain EVR. We have examined the impact of drug exposure on treatment efficacy [25–26] and reported that Peg-IFN is dose-dependently correlated with EVR [25]. In this study, the dose-dependent efficacy of Peg-IFN for EVR was also revealed in aged patients over 65 y.o., with less than 0.9 µg/kg/week of Peg-IFN leading to a low EVR rate for aged patients. If patients are difficult to treat with more than 1.2 µg/kg/week of Peg-IFN, using as much Peg-IFN as possible is desirable, in order to attain higher EVR rates. Accordingly, a reduction of Peg-IFN to 80% may need to be considered, although the manufacturer's drug information recommends reducing the dose of Peg-IFN to 50% of the assigned one. Since reduction of Peg-IFN has been reported to not affect the SVR rate after HCV RNA disappearance [26], using G-CSF for aged patients who develop severe neutropenia can be beneficial, especially in the first 12 weeks.

We also examined the negative prediction of SVR, i.e. an HCV RNA decrease at an earlier point of treatment than the usual prediction at treatment week 12 of a 2-log decrease, among aged patients with CH-C treated by Peg-IFN plus ribavirin combination therapy. We found that none of the patients without a 1-log decrease at week 4 or a 2-log decrease at week 8 could attain SVR, even if the complete treatment duration was given, the negative predictive value (NPV) for SVR equaled 100%. This earlier prediction is applied just as well to aged patients as to non-aged patients in order to avoid additional adverse effects. Recently, a genetic polymorphism near the *IL28B* gene has been reported to be associated with non-response to Peg-IFN plus ribavirin combination therapy [27–29], which is beneficial to patients. Nevertheless, in the presence of this genetic polymorphism, NPV for SVR remains at 57–87%; 100% accuracy is not guaranteed. Thus, in addition to the pretreatment prediction, an earlier negative prediction for SVR during treatment is also considered to be useful.

We have shown in this study that, in the presence of genotype 2, HCV was easily eliminated even among aged patients; the SVR rates were over 75% for patients who had completed the treatment, and these rates were similar up to 70 y.o. The SVR rate of genotype 2 patients over 70 y.o. was 43%, however, the age limitation of the treatment among patients over 70 y.o. remains unclear, because of the small number of patients enrolled in this study. We have reported that the reduction of treatment drugs had little effect on anti-viral efficacy for patients with genotype 2, meaning that SVR can be attained even with aged patients who are usually given lower drug doses than non-aged patients [30]. Patients under 70 y.o. with genotype 2 should, at least, benefit from this therapy. The SVR rate was maintained among genotype 2 patients being 65–69 y.o., compared to genotype 1 patients. The higher efficacy with shorter treatment duration in genotype 2 aged patients can account for it.

In conclusion, the strategy of a response-guided therapy and an earlier negative prediction for SVR may be beneficial for aged patients, especially those with genotype 1. At present, aged patients up to 65–70 y.o. with CH-C can be candidates for Peg-IFN plus ribavirin combination therapy, if its efficacy and adverse effects are fully taken into account. At the same time, there is an urgent need to establish new treatment procedures, such as combination therapy with protease inhibitor plus polymerase inhibitor without Peg-IFN or ribavirin, for non-responders or patients

with poor tolerability for Peg-IFN plus ribavirin combination therapy among aged patients.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this paper.

Acknowledgments

Other institutions and participants in the Osaka Liver Forum are: Higashiosaka City Central Hospital, S. Iio; Osaka Medical Center for Cancer and Cardiovascular Diseases, K. Imanaka; Kinki Central Hospital of Mutual Aid Association of Public School Teachers, E. Hayashi; Sumitomo Hospital, A. Yamada; Itami City Hospital, T. Kitada; Otemae Hospital, Y. Doi; Suita Municipal Hospital, T. Nagase; NTT West Osaka Hospital, A. Kaneko; National Hospital Organization Minami Wakayama Medical Center, K. Fujimoto; Nishinomiya Municipal Central Hospital, H. Ogawa; Saiseikai Senri Hospital, K. Suzuki; Osaka Kaisei Hospital, N. Imaizumi; Kano General Hospital, S. Kubota; Saso Hospital, M. Nishiuchi; and Meiwa Hospital, Y. Hayakawa.

This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from Ministry of Health Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses

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Received May 2009; accepted for publication June 2009

SUMMARY. Reducing the dose of drug affects treatment efficacy in pegylated interferon (Peg-IFN) and ribavirin combination therapy for patients with hepatitis C virus (HCV) genotype 1. The aim of this study was to investigate the impact of drug exposure, as well as the baseline factors and the virological response on the treatment efficacy for genotype 2 patients. Two-hundred and fifty patients with genotype 2 HCV who were to undergo combination therapy for 24 weeks were included in the study, and 213 completed the treatment. Significantly more patients who achieved a rapid virological response (RVR), defined as HCV RNA negativity at week 4, achieved a sustained virological response (SVR) (92%, 122/133) compared with patients who failed to achieve RVR (48%, 38/80) ($P < 0.0001$). Multivariate logistic-regression analysis showed that only platelet counts [odds ratio (OR), 1.68;

confidence interval (CI), 1.002–1.139] and RVR (OR, 11.251; CI, 5.184–24.419) were independently associated with SVR, with no correlation being found for the mean dose of Peg-IFN and ribavirin for RVR and SVR. Furthermore, in the stratification analysis of the timing of viral clearance, neither mean dose of Peg-IFN ($P = 0.795$) nor ribavirin ($P = 0.649$) affected SVR in each group. Among the patients with RVR, the lowest dose group of Peg-IFN ($0.77 \pm 0.10 \mu\text{g}/\text{kg}/\text{week}$) and ribavirin ($6.9 \pm 0.90 \text{mg}/\text{kg}/\text{day}$) showed 100% and 94% of SVR. Hence, RVR served as an important treatment predictor, and drug exposure had no impact on both SVR and RVR in combination therapy for genotype 2 patients.

Keywords: chronic hepatitis C, drug exposure, genotype 2, peginterferon and ribavirin combination therapy.

INTRODUCTION

The current standard of care for chronic hepatitis C (CHC) patients consists of combination therapy using pegylated

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CHC, chronic hepatitis C; c-EVR, complete early virological response; ETR, end of treatment response; γ -GTP, γ -glutamyl transpeptidase; HCV, hepatitis C virus; IFN, interferon; NPV, negative predictive value; Peg-IFN, pegylated interferon; RVR, rapid virological response; SVR, sustained virological response.

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interferon (Peg-IFN) and ribavirin [1–3]. Large, randomized clinical trials have demonstrated that 42–52% of hepatitis C virus (HCV) genotype 1 ‘difficult-to-treat’ patients achieved sustained virological response (SVR), whereas 76–84% of HCV genotype 2 or 3 infected patients treated with Peg-IFN and ribavirin achieved SVR [4–6]. It also has been shown that in HCV genotype 2 and 3 infected patients, 24-week treatment regimens are just as effective as 48-week regimens [6,7]. Therefore, current guidelines recommend a 24-week treatment for these patients in contrast to 48 weeks for genotype 1 patients [1–3]. However, as side effects are common and treatment is expensive for this therapy, it would be ideal to be able to further reduce the total amount of drug medication

without loss of treatment efficacy for genotype 2 and 3 patients.

In HCV genotype 1 patients, reducing drug doses affects treatment efficacy. In our investigation of HCV genotype 1 patients, the rate of complete early virological response (c-EVR), defined as HCV RNA negativity at week 12, was affected by the mean dose of Peg-IFN during the first 12 weeks dose-dependently ($P < 0.0001$) [8]. Furthermore, we showed that only 4% relapse was found in patients given ≥ 12 mg/kg/day of ribavirin among those with c-EVR, and the relapse rate showed a decline in relation to the increase in the dose of ribavirin ($P = 0.0002$) [9]. On the contrary, it remains to be determined whether treatment efficacy can be preserved by further reducing both drug doses in genotype 2 and 3 patients. Because lower doses are expected to cause fewer adverse effects, it is important to find whether reduced drug doses can be used while retaining efficacy.

In the present study, we retrospectively evaluated the efficacy of Peg-IFN alpha-2b and ribavirin combination therapy for 24 weeks in patients infected with HCV genotype 2 and analysed the factors that affected the treatment efficacy, with particular interests in the drug impact of Peg-IFN and ribavirin.

PATIENTS AND METHODS

Patient selection and study design

Patients considered to be eligible for this study were those infected with HCV genotype 2 who underwent Peg-IFN alpha-2b (Schering-Plough K.K., Tokyo, Japan) and ribavirin (Schering-Plough K.K.) combination therapy from December 2005 to July 2007 at 29 medical institutions taking part in the Osaka Liver Forum and had completed the 24-week observation after a clinical course of 24 weeks. Patients with the following criteria were excluded: hepatitis B virus or human immunodeficiency virus coinfection, decompensated liver disease, severe cardiac, renal, haematological or chronic pulmonary disease, poorly controlled psychiatric disease, poorly controlled diabetes and immunologically mediated disease. Liver biopsy had been performed within 24 months prior to the treatment, and histological results were classified according to the METAVIR scoring system [10].

Written informed consent was obtained from each patient, and the study protocol was reviewed and approved according to the ethical guidelines of the 1975 Declaration of Helsinki by institutional review boards at the respective sites.

Patients were treated with Peg-IFN alpha-2b plus ribavirin for the duration of the study of 24 weeks. Peg-IFN alpha-2b and ribavirin dosages were based on body weight according to the manufacturer's instructions: Peg-IFN alpha-2b was given subcutaneously weekly (45 kg or less, 60 μ g/dose; 46–60 kg, 80 μ g/dose; 61–75 kg, 100 μ g/dose; 76–90 kg,

120 μ g/dose; 91 kg or more, 150 μ g/dose), and ribavirin was given orally daily (60 kg or less, 600 mg/day; 61–80 kg, 800 mg/day; 81 kg or more, 1000 mg/day). The drug doses were also modified based on the manufacturer's instructions according to the intensity of the haematologic adverse effects.

Virological tests

Serum HCV RNA level was quantified by PCR assay (COBAS Amplicor HCV Test v2.0, Chugai-Roche Diagnostics, Tokyo, Japan), with a sensitivity limit of 5000 IU/mL and a dynamic range from 5000 to 5 000 000 IU/mL [11].

Serum HCV RNA was assessed by qualitative PCR assay (COBAS Amplicor HCV Monitor Test v2.0, Chugai-Roche Diagnostics), with a detection limit of 50 IU/mL [12].

Assessment of efficacy

Serum HCV RNA (qualitatively or quantitatively) was measured at weeks 4, 8, 12 and 24 during treatment and after 24 weeks of follow-up without treatment. Patients were classified as having a rapid virological response (RVR) if serum HCV RNA was undetectable (< 50 IU/mL) at week 4 and at the end of treatment response (ETR) at week 24 of treatment. SVR was defined as undetectable HCV RNA at week 24 after treatment. Patients with an ETR who sero-reverted to HCV RNA during follow-up were classified as relapsers.

Drug exposure

The amounts of Peg-IFN alpha-2b and ribavirin actually taken by each patient during the treatment period were evaluated by reviewing the medical records. The mean doses of both drugs were calculated individually as averages on the basis of body weight at baseline; Peg-IFN alpha-2b expressed as μ g/kg/week and ribavirin as mg/kg/day.

Data collection

The medical records were retrospectively reviewed and the factors necessary for this examination were extracted: age, sex, body weight, body mass index (BMI), basic laboratory assessments, liver histology, quantitative and qualitative HCV RNA, dose of Peg-IFN alpha-2b and ribavirin received at each administration, and the response to treatment.

Statistical analysis

This study was a retrospective study and, for treatment results and the analysis of related factors, analysis was carried out only for cases in which the treatment had been completed (per-protocol analysis). Continuous variables are reported as the mean with standard deviation (SD) or

median level, while categorical variables are shown as the count and proportion. In univariate analysis, the Mann-Whitney *U*-test was used to analyse continuous variables, while chi-squared and Fisher's exact tests were used for analysis of categorical data. Variables with $P < 0.05$ at univariate analysis were retained for the multivariate logistic-regression analysis. Stepwise and multivariate logistic-regression models were used to explore the independent factors that could be used to predict a virological response. The significance of trends in values was determined with the Mantel-Haenszel chi-square test. For all tests, two-sided *P*-values were calculated and the results were considered statistically significant if $P < 0.05$. Statistical analysis was performed using the SPSS program for Windows, version 15.0J (SPSS, Chicago, IL, USA).

RESULTS

The baseline characteristics for the total cohort are shown in Table 1. Most of the patients were female (56%) with a mean age of 54 years. Seventy per cent of the patients were treatment naïve. Of the 250 patients, liver biopsies were performed for 174 patients, and 18 of them had advanced fibrosis (F 3–4).

Of the total of 250 patients, 37 (15%) were withdrawn from treatment because of adverse events: decreased haemoglobin ($n = 10$), psychiatric problems including depression ($n = 9$), fatigue ($n = 3$), thrombocytopenia, neutropenia, pyrexia, rash, cerebral haemorrhage, bleeding of ocular fundus, dyspnea, dizziness, jaundice, transaminase rise, gastrointestinal symptoms ($n = 1$) and other adverse

events ($n = 4$). Eight of these patients who discontinued treatment prematurely had SVR (8/37; 22%).

Drug adherence

Seventy-nine of the 213 patients (37%) required dose reduction of Peg-IFN alpha-2b, 99 (46%) of ribavirin because of adverse events (not including patients who later discontinued treatment because of adverse event). Neutropenia (24/79; 30%) and thrombocytopenia (24/79; 30%) were the most common adverse events for dose reduction of Peg-IFN alpha-2b, and decreased haemoglobin (82/99; 83%) for that of ribavirin.

Virological response

Of the 213 patients who completed 24 weeks of treatment and 24 weeks of follow-up, 160 (75%) patients were clear of HCV RNA at week 4, 191 (90%) at week 8, 196 (92%) at week 12. ETR was observed for 195 (92%), and SVR for 160 (75%). The relapse rate was 18% (35/195).

Virological response according to the timing of viral clearance

Positive and negative prediction of sustained virological response according to the timing of viral clearance

We examined SVR rates according to the timing of viral clearance for the cases in which HCV RNA was cleared during the treatment (Fig. 1a). The SVR rate was 92% (122/133) for patients clear of HCV RNA until week 4, 64% (37/58) from week 5 until week 8, 20% (1/5) from week 9 until

Number of cases	250	
Age (years)*	54.0 ± 12.4	(22–76)
Sex (male/female)	110/140	
Body weight (kg)*	60.3 ± 11.7	(39–99)
Body mass index (kg/m ²)*	23.1 ± 3.2	(16–35)
Past IFN therapy (naïve/experienced)†	175/70	
HCV RNA (KIU/mL)‡	1700	(4–5000 <)
Fibrosis (0/1/2/3/4)§	18/98/40/14/4	
Activity (0/1/2/3)§	15/81/70/8	
White blood cells (/mm ³)*	5210 ± 1,750	(2100–13 870)
Neutrophils (/mm ³)*	2700 ± 1,250	(590–9020)
Red blood cells (×10 ⁴ /mm ³)*	436 ± 48	(307–554)
Haemoglobin (g/dL)*	13.9 ± 1.4	(10–18)
Platelets (×10 ⁴ /mm ³)*	18.3 ± 6.4	(4–41)
ALT (IU/L)*	79 ± 77	(13–581)
γ-GTP (U/L)*	56 ± 65	(7–479)
Creatinine(mg/dL)*	0.7 ± 0.1	(0.4–1.1)

Table 1 Baseline demographic and viral characteristics of patients

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase. *Values expressed as mean ± SD (range), †interferon treatment history was not known for five patients, ‡values expressed as median (range), §data for 76 patients are missing.

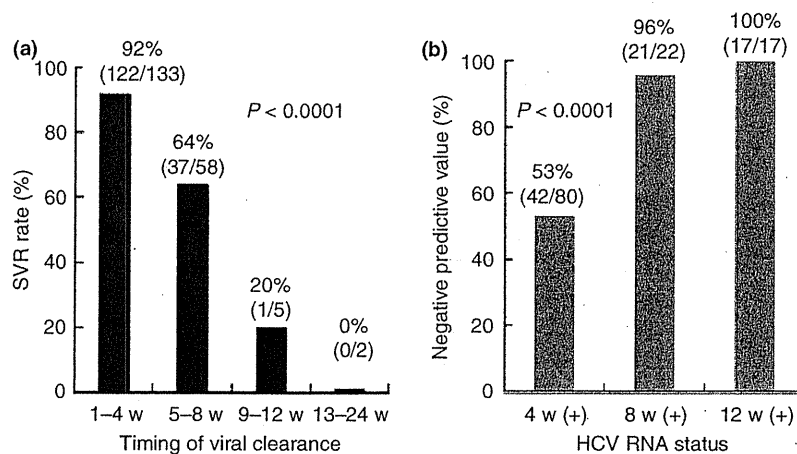


Fig. 1 (a) SVR rates according to timing of viral clearance. The number above each bar shows the percentage, and the numbers inside parentheses show the number of patients showing responses over the total number in the subgroup. The timing of viral clearance was time-dependently correlated with SVR ($P < 0.0001$). (b) Negative predictive values according to time of HCV RNA positivity. The number above each bar shows the percentage, and the numbers inside parentheses show the number of patients showing responses over the total number in the subgroup. The time of HCV RNA positivity was time-dependently correlated with NPV ($P < 0.0001$).

week 12 and 0% (0/2) from week 13 until week 24. The Mantel-Haenszel chi-square test showed that SVR rates were diminished with a delay in the timing of viral clearance becoming late ($P < 0.0001$). Significantly, more patients who attained RVR achieved final SVR (92%, 122/133) than patients who failed to attain RVR (48%, 38/80; $P < 0.0001$).

Next, we examined the negative predictive value (NPV) for the proportion of patients with treatment failure among those with HCV RNA persistence at week 4, 8 and 12 (Fig. 1b). NPV was 53% at week 4, 96% at week 8 and 100% at week 12. Only one of the 22 patients with positive HCV RNA at week 8 reached SVR.

Predictors of sustained virological response

Both pretreatment and treatment factors that could be associated with the response to Peg-IFN and ribavirin combination therapy were compared between patients with and without SVR in Table 2. This univariate analysis showed that age ($P = 0.029$), baseline HCV RNA level ($P = 0.033$), past IFN treatment history ($P = 0.028$), platelets counts ($P = 0.020$) and having RVR ($P < 0.0001$) contributed to achievement of SVR. Factors that were significantly associated with SVR by univariate analysis were then analysed by multivariate logistic regression analysis. SVR was attained independent of high platelet counts [odds ratio (OR) 1.070, 95% confidence interval (CI) 1.003–1.140, $P = 0.040$] and having RVR (OR 11.526, 95% CI 5.317–24.984, $P < 0.0001$; Table 3). As for drug doses, the mean dose of Peg-IFN alpha-2b was $1.32 \pm 0.27 \mu\text{g/kg/week}$ in patients with SVR and $1.27 \pm 0.29 \mu\text{g/kg/week}$ in those without

SVR ($P = 0.130$), while that of ribavirin was 10.2 ± 1.9 and $10.2 \pm 2.0 \text{ mg/kg/day}$ ($P = 0.949$), respectively. Thus, neither Peg-IFN nor ribavirin drug exposure during the full treatment period affected attainment of SVR.

Predictors of rapid virological response

To delineate features that might help identify patients most likely to reach RVR, we also analysed these factors because having RVR turned out to be one of the most powerful predictors of SVR attainment. By univariate and multivariate logistic-regression analyses, RVR was attained independent of younger age (OR 0.648, 95% CI 0.494–0.850, $P = 0.002$) and lower baseline HCV RNA level (OR 0.964, 95% CI 0.944–0.984, $P < 0.0001$; Tables 4 & 5). The mean dose of Peg-IFN alpha-2b during the first 4 weeks was $1.31 \pm 0.27 \mu\text{g/kg/week}$ in patients with RVR and $1.31 \pm 0.29 \mu\text{g/kg/week}$ in those without RVR ($P = 0.259$), that of ribavirin was $10.1 \pm 1.8 \text{ mg/kg/day}$ and $10.3 \pm 2.1 \text{ mg/kg/day}$ ($P = 0.637$), respectively. Thus, neither Peg-IFN nor ribavirin drug exposure during the first 4 weeks had an impact on attainment of RVR.

Virological response according to drug exposure and the timing of viral clearance

Impact of drug exposure on sustained virological response

To more closely evaluate the impact of drug exposure on virological response, we classified the average doses of both drugs into four categories (Peg-IFN alpha-2b: up to $0.9 \mu\text{g/kg/week}$, from 0.9 to $>1.2 \mu\text{g/kg/week}$, from 1.2 to $>1.5 \mu\text{g/kg/week}$, from $1.5 \mu\text{g/kg/week}$; ribavirin: up to

Factor	SVR (n = 160)	Non-SVR (n = 53)	P-value
Age (years)*	52.4 ± 12.6	56.9 ± 10.2	0.029
Sex (male/female)	66 / 94	26 / 27	0.202
Body weight (kg)*	59.5 ± 11.5	59.9 ± 12.5	0.896
Body mass index (kg/m ²)*	22.8 ± 3.1	22.8 ± 3.5	0.817
HCV RNA (KIU/mL) [†]	1170	1600	0.033
Past IFN therapy (naive/experienced) [‡]	116/41	31/22	0.028
Fibrosis (F 0–2/3–4) [§]	106/10	30/5	0.247
Activity (A 0–1/2–3) [§]	62/54	20/15	0.847
White blood cells (/mm ³)*	5260 ± 1680	4720 ± 1500	0.078
Neutrophils (/mm ³)*	2740 ± 1270	2420 ± 1020	0.186
Red blood cells (×10 ⁴ /mm ³)*	435 ± 44	437 ± 55	0.820
Haemoglobin (g/dL)*	13.9 ± 1.3	14.0 ± 1.5	0.441
Platelets (×10 ⁴ /mm ³)*	19.0 ± 6.0	16.5 ± 6.2	0.020
ALT (IU/L)*	86 ± 89	64 ± 45	0.514
γ-GTP (U/L)*	54 ± 67	58 ± 59	0.512
Creatinine (mg/dL)*	0.7 ± 0.1	0.7 ± 0.1	0.457
Mean Peg-IFN dose (μg/kg/week)*	1.32 ± 0.27	1.27 ± 0.29	0.130
Mean ribavirin dose (mg/kg/day)*	10.2 ± 1.9	10.2 ± 2.0	0.949
RVR (yes/no)	122/11	38/42	<0.0001

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; CI, confidence interval. *Values expressed as mean ± sd, [†]values expressed as median, [‡]interferon treatment history was not known for three patients, [§]data for 62 patients are missing.

Factor	Category	Odds ratio	95% CI	P-value
Age (years)	By 10	–	–	NS
HCV RNA (KIU/mL)	By 100 KIU/mL	–	–	NS
Platelets (×10 ⁴ /mm ³)	By 1 × 10 ⁴ /mm ³	1.068	1.002–1.139	0.045
Past IFN therapy	Naive/experienced	–	–	NS
RVR	Yes/no	11.251	5.184–24.419	<0.0001

IFN, interferon; HCV, hepatitis C virus; CI, confidence interval.

8 mg/kg/day, from 8 to >10 mg/kg/day, from 10 to >12 mg/kg/day, from 12 mg/kg/day). SVR rates relative to the mean drug doses during the full treatment period and the timing of HCV RNA clearance are shown in Table 6. As also shown in Fig. 1a, the respective rates for SVR according to the timing of viral clearance were 92% in patients clear of HCV RNA until week 4, 64% from week 5 until week 8 and 14% from week 9 until week 24. On the contrary, according to mean drug doses, the respective rates for SVR were 89% (24/27), 73% (11/15), 79% (85/107) and 82% (40/49) in patients who received Peg-IFN up to 0.9 μg/kg/week, from 0.9 to >1.2 μg/kg/week, from 1.2 to >1.5 μg/kg/week and from 1.5 μg/kg/week, respectively, and 80% (24/30), 80% (40/50), 82% (68/83) and 79% (27/34) in patients who received ribavirin up to 8 mg/kg/day, from 8 to >10 mg/kg/day, from 10 to >12 mg/kg/day and from 12 mg/kg/day,

respectively. If the category of the timing of viral clearance was the same, the respective rates for SVR attainment according to the mean doses of both Peg-IFN and ribavirin were similar. Furthermore, multivariate analysis by the Mantel-Haenszel chi-square test showed that neither the mean dose of Peg-IFN ($P = 0.795$) nor ribavirin ($P = 0.649$) affected SVR rates after stratification of the timing of viral clearance. Among the patients with RVR, SVR rates were as high as 88–100% regardless of Peg-IFN alpha-2b medication, and the least medicated group (<0.9 μg/kg/week, the mean dose with SD was 0.77 ± 0.10 μg/kg/week, 0.50–0.89) showed 100% of SVR rate (19/19). Similarly, SVR rates were as high as 91–94% regardless of ribavirin medication among the patients with RVR, and 17 of 18 patients (94%) in the least medicated group (<8 mg/kg/day, the mean dose with SD was 6.9 ± 0.90 mg/kg/day, 5.0–7.9)

Table 2 Factors associated with SVR among patients who completed the treatment – univariate analysis

Table 3 Factors associated with SVR among patients who completed the treatment – multivariate analysis

Table 4 Factors associated with RVR among patients who completed the treatment – univariate analysis

Factor	RVR (n = 133)	Non-RVR (n = 80)	P-value
Age (years)*	51.9 ± 12.3	56.3 ± 11.3	0.010
Sex (male/female)	60/73	32/48	0.279
Body weight (kg)*	60.2 ± 11.6	58.6 ± 11.9	0.276
Body mass index (kg/m ²)*	22.9 ± 3.2	22.6 ± 3.1	0.369
HCV RNA (KIU/mL) [†]	1050	1800	0.001
Past IFN therapy (naive/experienced) [‡]	97/34	50/29	0.068
Fibrosis (F 0–2/3–4) [§]	86/8	50/7	0.315
Activity (A 0–1/2–3) [§]	51/43	31/26	1.000
White blood cells (per mm ³)*	5300 ± 1760	4850 ± 1400	0.205
Neutrophils (per mm ³)*	2740 ± 1290	2530 ± 1090	0.340
Red blood cells (×10 ⁴ /mm ³)*	440 ± 45	432 ± 49	0.628
Haemoglobin (g/dL)*	13.9 ± 1.4	13.9 ± 1.4	0.975
Platelets (×10 ⁴ /mm ³)*	18.9 ± 6.1	17.5 ± 6.1	0.170
ALT (IU/L)*	87 ± 93	69 ± 52	0.630
γ-GTP (U/L)*	57 ± 71	53 ± 53	0.658
Creatinine (mg/dL)*	0.7 ± 0.1	0.7 ± 0.1	0.203
Mean Peg-IFN dose (µg/kg/week)*	1.31 ± 0.27	1.31 ± 0.29	0.259
Mean ribavirin dose (mg/kg/day)*	10.1 ± 1.8	10.3 ± 2.1	0.637

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; CI, confidence interval. *Values expressed as mean ± SD, [†]values expressed as median, [‡]interferon treatment history was not known for three patients, [§]data for 62 patients are missing.

Table 5 Factors associated with RVR among patients who completed the treatment – multivariate analysis

Factor	Category	Odds		P-value
		ratio	95% CI	
Age (years)	By 10	0.648	0.494–0.850	0.002
HCV RNA (KIU/mL)	By 100 KIU/mL	0.964	0.944–0.984	<0.0001

HCV, hepatitis C virus; CI, confidence interval.

achieved SVR. In addition, we examined the drug impact on SVR in the patients with the least medication of both drugs (<0.9 µg/kg/week of Peg-IFN and <8 mg/kg/day of ribavirin). Nine patients were categorized into this group and six of these patients achieved SVR (67%); patients with RVR had a significantly higher SVR rate (100%, 5/5) than patients without RVR (25%, 1/4; $P = 0.048$). Thus, SVR attainment was dependent on time, not on drug dose.

DISCUSSION

In the present study, we found that having RVR and high platelet counts were statistically associated with reaching SVR according to multivariate analysis. The timing of viral clearance was closely related to the treatment effect in

patients with genotype 2, similar to the case for those with genotype 1. Ninety-two per cent of SVR was observed for patients with RVR and, conversely, 96% of the patients with HCV RNA positivity at week 8 showed non-SVR. The predictability of SVR based on EVR, defined as a decline of at least 2-log from the baseline of the HCV RNA level at week 12, has been assessed, and genotype 1 patients who have failed to reach EVR are recommended to discontinue the treatment after 12 weeks, because the likelihood of SVR is 0–3% in the absence of EVR [5,13]. On the basis of our examination of patients with genotype 2, not EVR, but 8-week monitoring of the HCV RNA level can be used.

As a significant factor for SVR, not liver fibrosis, but the platelet count was selected. Everson *et al.* [14] reported that patients with low platelet counts ($\leq 12.5 \times 10^4/\text{mm}^3$) achieved lower SVR rates than patients with normal platelet counts ($> 12.5 \times 10^4/\text{mm}^3$) even in the case of patients with the same category of liver fibrosis treated by Peg-IFN plus ribavirin combination therapy. Thus, independent of liver fibrosis, thrombocytopenia itself seems to participate in treatment failure, although the mechanism remains unknown.

Our study also demonstrated that younger age (OR 0.648, 95% CI 0.494–0.850, $P = 0.002$) and lower HCV RNA level (OR 0.964, 95% CI 0.944–0.984, $P < 0.0001$) were statistically associated with reaching an RVR. Zeuzem *et al.* [7] previously reported that pretreatment viral load was not

Table 6 SVR rates according to Peg-IFN alpha-2b and ribavirin exposure and the timing of viral clearance among patients with virological response during the treatment

Timing of viral clearance (week)	Peg-IFN dose ($\mu\text{g}/\text{kg}/\text{week}$)				Ribavirin dose ($\text{mg}/\text{kg}/\text{day}$)				Total
	<0.9	0.9–1.2	1.2–1.5	1.5 \leq	<8	8–10	10–12	12 \leq	
1–4	100% (19/19)	91% (10/11)	92% (65/71)	88% (28/32)	94% (17/18)	92% (33/36)	91% (51/56)	91% (20/22)	92% (122/133)
5–8	63% (5/8)	33% (1/3)	64% (19/30)	71% (12/17)	58% (7/12)	54% (7/13)	74% (17/23)	60% (6/10)	64% (37/58)
9–24	–	0% (0/1)	17% (1/6)	–	–	0% (0/1)	0% (0/4)	50% (1/2)	14% (1/7)
Total	89% (24/27)	73% (11/15)	79% (85/107)	82% (40/49)	80% (24/30)	80% (40/50)	82% (68/83)	79% (27/34)	81% (160/198)

* $P = 0.795$ for comparison of the four Peg-IFN groups after stratification of the timing of viral clearance. ** $P = 0.649$ for comparison of the four ribavirin groups after stratification of the timing of viral clearance.

associated with reaching RVR in genotype 2 patients. In contrast, Dalgard *et al.* [15] reported that independent predictors of RVR in genotype 2 or 3 patients were male gender, younger age (≤ 40 years) and low viral load (≤ 400 KIU/mL). The influence of viral load on reaching RVR remains controversial in the Peg-IFN and ribavirin combination therapy in genotype 2 patients, but patients with lower viral load seem favoured to reach HCV RNA levels below the detection limit, that is, to attain RVR, if the virological response is the same.

Recently, because of substantial adverse effects and costs associated with this therapy, studies have been carried out to determine the possibility of further reducing the total amount of drug medication without compromising antiviral efficacy in HCV genotype 2 and 3 patients. There seem to be two ways to achieve. One is by shortening the treatment duration, and the other is by decreasing the doses of the treatment drugs. With respect to the former, several studies on genotype 2 patients have been reported. At first, some studies of small numbers of subjects demonstrated that cumulatively analysed genotype 2 and 3 patients had high SVR rates up to 12 to 16 weeks of therapy (82–94%), similar to patients subjected to 24-week therapy (76–95%) [16–19]. However, further prospective investigation of large numbers of subjects revealed that shortening the treatment duration was associated with an increase in the rate of relapse and that significantly higher relapse rates led to lower SVR rates (71–81.1%), even among those with RVR [15,20,21]. The latest study by Mangia *et al.* [22] showed that shortened therapy after RVR was acceptable only for patients who had no signs of advanced liver fibrosis and low BMI. Considering the results of these trials, shortened therapy is regarded as optional treatment for selected patients displaying favourable baseline characteristics. Therefore, shortening treatment duration from 24 weeks should not be generally recommended for patients who are infected genotype 2 or 3 and can tolerate 24-week Peg-IFN and ribavirin combination therapy.

Another attempt to improve the treatment tolerability for genotype 2 or 3 patients has focused on dose reduction of treatment drugs. Weiland *et al.* [23] examined low-dose Peg-IFN alpha-2a (135 μg weekly) with a weight-based standard-dose of ribavirin (11 mg/kg daily) for genotype 2 and 3 patients. They demonstrated that SVR rates of 86% were achieved, which is equal to those in previous representative randomized controlled studies of standard dose Peg-IFN therapy (76–84%) [4–6]. In contrast, Ferenci *et al.* [24] examined the efficacy of standard-dose Peg-IFN alpha-2a (180 μg weekly) with low-dose ribavirin (400 mg daily) in comparison with standard-dose Peg-IFN alpha-2a (180 μg weekly) and ribavirin (800 mg daily) for genotype 2 and 3 patients, and demonstrated that there was no difference between the two treatment groups with respect to SVR rates (64% with 400 mg/day compared with 69% with 800 mg/day) and relapse rates (20% with 400 mg/day compared

with 17% with 800 mg/day). These studies showed that either drug dose can be reduced for genotype 2 and 3 patients without compromising antiviral efficacy. In the present study, neither Peg-IFN nor ribavirin drug exposure participated in reaching RVR and SVR. In particular, more than 90% of patients having RVR achieved SVR regardless of the drug exposure level, as long as the mean Peg-IFN dose was over 0.5 µg/kg/week and ribavirin was over 5.0 mg/kg/day. The results of our study suggested that genotype 2 patients may receive reduced levels of both drug doses on the condition that they can complete the full 24-week course of combination therapy. Randomized, prospective trials that reduced both Peg-IFN and ribavirin should be conducted for CHC patients to clarify this.

In the present study, while the treatment outcome was independent of the individual ribavirin exposure in patients who had completed the 24-week treatment, the most common reason to withdraw the treatment was decreased haemoglobin because of ribavirin medication. Based on the results of randomized controlled trials [6], using a ribavirin dose of 800 mg/day is recommended for genotype 2/3 patients [1–3]. However, several studies have shown that some patients cannot tolerate even this suboptimal ribavirin dose. This is a serious problem for patients with the risk of anaemia, especially elderly patients. The ageing of patients is progressing around the world, requiring improvement in treatment tolerability. Recently, Andriulli *et al.* [25] examined the effect of ribavirin in a 12-week course of therapy on CHC genotype 2 patients with RVR in two groups; one continuing with ribavirin and the other receiving Peg-IFN alpha-2a alone after week 6. The relapse rates were higher (46% vs 17%; $P < 0.001$) and overall SVR rates were lower (54 vs 82%; $P < 0.001$) in patients who stopped receiving ribavirin at week 6. Thus, ribavirin medication throughout the treatment period is necessary to raise the SVR rate even in genotype 2 or 3 patients with RVR. In the present study, the ribavirin dose could be reduced without loss of efficacy for genotype 2 patients, as long as the patients were treated for 24 weeks. Therefore, in the patients with the risk of anaemia, it would be better to reduce the dose of ribavirin before anaemia arises rather than being forced to discontinue the combination therapy because of anaemia caused by ribavirin medication. We previously reported that in CHC patients treated by IFN or Peg-IFN in ribavirin combination therapy, a decline of haemoglobin concentration by 2 g/dL at the end of 2 weeks from the start of the treatment can be used to identify patients likely to develop severe anaemia [26,27]. This kind of predictive factor for the progression to severe anaemia can be of much help in reducing ribavirin with appropriate timing.

Our study has some limitations. First, it is a retrospective study, and we could not obtain complete information for all patients. However, this is the first study of Peg-IFN and ribavirin combination therapy in which the drug dose of Peg-IFN and ribavirin taken by each patient was assessed

independently for HCV genotype 2 patients. Our results can be taken as an evidence offering suggestions for the treatment of CHC genotype 2 patients. Second, this cohort included patients with different histories of past IFN treatment. Patients who had failed to recover with previous IFN-based treatment were likely to experience treatment failure again [28]. Therefore, we examined the predictors of treatment response separately according to treatment history, and confirmed that in both naïve and treatment-experienced patients, the mean dose of Peg-IFN and ribavirin showed no correlation with SVR or RVR in both groups.

In conclusion, our study demonstrates that RVR is an important treatment predictor and more than 90% of patients having RVR achieve SVR with combination therapy of Peg-IFN and ribavirin for genotype 2 infected CHC patients regardless of the drug exposure. Further prospective, randomized studies are necessary to assess whether the standard or a reduced dose of each drug can produce equivalent outcomes.

ACKNOWLEDGEMENTS

Other institutions and participants in the Osaka Liver Forum are: Hyogo Prefectural Nishinomiya Hospital, Y. Inui; Osaka Medical Center for Cancer and Cardiovascular Diseases, K. Katayama, K. Imanaka; National Hospital Organization Osaka Minami Medical Center, T. Hijioka; Sumitomo Hospital, A. Yamada; Osaka Koseinenkin Hospital, T. Ito; Suita Municipal Hospital, T. Nagase; Itami City Hospital, T. Kashiwara; Otemae Hospital, Y. Doi; Ashiya Municipal Hospital, K. Kiriyama; NTT West Osaka Hospital, A. Kaneko; Osaka Kaisei Hospital, N. Imaizumi; Nishinomiya Municipal Central Hospital, H. Ogawa; Kano General Hospital, S. Kubota; and Saiseikai Senri Hospital, K. Suzuki. This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from Ministry of Health Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science and Culture of Japan.

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Amino Acid Substitution in the Core Protein has no Impact on Relapse in Hepatitis C Genotype 1 Patients Treated With Peginterferon and Ribavirin

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Previous reports demonstrated that amino acid (aa) substitutions in the hepatitis C virus (HCV) core protein are predictors of non-virological responses to pegylated interferon (Peg-IFN) and ribavirin combination therapy. The aim of this study was to investigate the impact of core aa substitutions on viral kinetics during the treatment and relapse after the treatment. The 187 patients with HCV genotype 1 enrolled in this study were categorized into four groups according to core aa substitution patterns: double-wild group (n=92), Arg70/Leu91; 70-mutant group (n=42), Gln70/Leu91; 91-mutant group (n=31), Arg70/Met91; and double-mutant group (n=22), Gln70/Met91. The relationship between the core aa substitutions and the virological response was examined. Multivariate logistic regression analyses showed that substitution at aa 70 was significantly associated with a poor virological response during the first 12 weeks (decline of <1 log from baseline at week 4, <2 log at week 12), and substitution at aa 91 was significantly associated with detectable HCV RNA at week 24. With respect to relapse, only the ribavirin exposure (odds ratio (OR), 0.77; 95% confidence interval (CI), 0.60–0.98) and HCV RNA disappearance between weeks 13 and 24 (OR, 23.69; 95% CI, 5.44–103.08) were associated independently with relapse, with no correlation being found with the core aa substitutions and relapse. In conclusion, the results showed that core aa substitutions can be strong predictive factors at pretreatment of the non-response, but not for relapse, for virological responders with HCV RNA disappearance during treatment. **J.**

Med. Virol. 83:419–427, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: amino acid substitution; core protein; hepatitis C virus; peginterferon and ribavirin combination therapy; relapse

INTRODUCTION

The current standard of care for chronic hepatitis C patients is combination therapy using pegylated interferon (Peg-IFN) and ribavirin [Anonymous, 2002; Strader et al., 2004; Dienstag and McHutchison, 2006]. However, the treatment outcome in response to this combination therapy among patients infected with hepatitis C virus (HCV) genotype 1 is still unsatisfactory and the chance of sustained virological response ranges from 42% to 52% [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. Therefore, tailoring treatment regimens for individual patients has become an important issue.

Grant sponsor: Ministry of Health Labor and Welfare of Japan (Research on Hepatitis and BSE); Grant sponsor: Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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Accepted 20 September 2010

DOI 10.1002/jmv.21975

Published online in Wiley Online Library (wileyonlinelibrary.com).

Outcome of therapy is influenced by various factors. Some host factors, such as age, sex, body weight, insulin resistance, and liver fibrosis have been reported as pretreatment factors affecting virological response to this combination therapy [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004; Romero-Gómez et al., 2005]. Recently, several genome-wide association studies identified single nucleotide polymorphisms (SNPs) near the interleukin (IL)-28B gene, which encodes interferon (IFN) lambda-3, as associated with response to Peg-IFN plus ribavirin treatment among patients infected with HCV of European [Suppiah et al., 2009], African [Ge et al., 2009], and Asian ancestry [Tanaka et al., 2009]. These studies suggest that host genetic variants may be associated strongly with response to IFN-alpha-based therapy. However, the ethical problem to perform host genetic search for all patients remains, and the sustained virological response rate is only 48–69% in patients having favorable IL-28B genotype to this combination therapy [Thompson et al., 2010].

Response-guided therapy is a dynamic approach to management of chronic hepatitis C patients based on the virological response at weeks 4 and 12 of treatment. At present, it is regarded as an excellent strategy for optimizing the treatment duration for individual patients. Earlier HCV RNA disappearance has been shown to lead to a higher sustained virological response rate [Ferenci et al., 2005; Berg et al., 2006; McHutchison et al., 2009], while patients without an early virological response, defined as showing an at least 2 log decrease from the baseline of HCV RNA levels at week 12 is recommended for discontinuing the treatment under the current guidelines [Anonymous, 2002; Strader et al., 2004; Dienstag and McHutchison, 2006].

In addition to viral kinetics during treatment, other viral factors have also been reported to be associated with this combination therapy outcome [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004; Shirakawa et al., 2008]. Previous studies indicated that amino acid (aa) 70 and/or 91 substitutions in the HCV core protein were independent pretreatment predictors of null or weak response to this combination therapy in genotype 1 patients [Akuta et al., 2007b,c]. The HCV core protein has been reported to inhibit signal transducer and activator of transcription (STAT)-1 phosphorylation, and disrupt the normal IFN-stimulated transcriptional response to viral infection [Lin et al., 2006]. It is supposed that the HCV core region might be associated with resistance to IFN therapy involving the Janus activated kinase (Jak)-STAT signaling cascade [Blindenbacher et al., 2003; Bode et al., 2003; Melen et al., 2004; de Lucas et al., 2005]. Recently, Okanoue et al. [2009] have demonstrated that wild type of core aa 70 and 91 are important for positive prediction of the virological response. However, the impact of core aa substitutions on the extent of HCV RNA decline during the treatment or virological relapse after completion of treatment has not yet been investigated in detail. Approximately 30% of genotype 1 patients who become

HCV RNA negative at the end of the treatment will experience relapse [Hadziyannis et al., 2004]. Being able to distinguish between end-of-treatment responders with a high probability of relapse and those with a low probability of relapse will be useful in reducing relapse rates and improving treatment outcome.

The aim of this study was to evaluate the impact of aa substitutions in the HCV core protein on viral kinetics and virological relapse in patients with HCV genotype 1 treated by Peg-IFN alpha-2b and ribavirin combination therapy.

PATIENTS AND METHODS

Patient Selection and Study Design

Patients considered to be eligible for this study were those who were infected with HCV genotype 1, had a viral load more than 10^5 IU/ml, had started Peg-IFN alpha-2b (Schering-Plough K.K. Tokyo, Japan) and ribavirin (Schering-Plough K.K.) combination therapy from December 2005 to June 2008 at Osaka University Hospital and three other medical institutions taking part in the Osaka Liver Forum, and had been examined with respect to the aa sequences at positions 70 and 91 in the HCV core protein with pretreatment serum samples. Patients with the following criteria were excluded: hepatitis B virus or human immunodeficiency virus co-infection; decompensated liver disease; severe cardiac, renal, hematological, or chronic pulmonary disease; poorly controlled psychiatric disease; poorly controlled diabetes; and immunologically mediated disease. As a result of screening at the institutions concerned, 187 patients with HCV genotype 1 were enrolled in this study. Liver biopsy had been performed within 12 months prior to the treatment, and histological results were classified according to the METAVIR scoring system [Bedossa and Poynard, 1996].

Written informed consent was obtained from each patient, and the study protocol was reviewed and approved according to the ethical guidelines of the 1975 Declaration of Helsinki by Institutional Review Boards at the respective sites.

Peg-IFN alpha-2b and ribavirin dosages were based on body weight according to the manufacturer's instructions: Peg-IFN alpha-2b was given subcutaneously weekly (45 kg or less, 60 µg/dose; 46–60 kg, 80 µg/dose; 61–75 kg, 100 µg/dose; 76–90 kg, 120 µg/dose; and 91 kg or more, 150 µg/dose), and ribavirin was given orally daily (60 kg or less, 600 mg/day; 61–80 kg, 800 mg/day; and 81 kg or more, 1,000 mg/day). The drug doses were also modified based on the manufacturer's instructions according to the severity of the adverse hematologic effects.

Detection of Amino Acid Substitutions in Core Region

The nucleotide sequence encoding aa 1–191 (the core protein of HCV) was analyzed by direct sequencing as described by Akuta et al. [2005, 2007b]. In brief, HCV