Keywords Hepatitis C virus · Relapse · Pegylated interferon · Ribavirin

Introduction

Chronic hepatitis C virus (HCV) infection is a major cause of viral hepatitis. An estimated 170 million people worldwide are infected with persistent HCV, and cirrhosis and complications of endstage liver disease will develop in many of these people [1]. Previous studies have shown that interferon (IFN) is among the most frequently used agents against HCV infection and is effective for eradicating HCV [2, 3] and that it significantly reduces the progression of liver fibrosis and the risk of hepatocellular carcinoma [4, 5]. Currently, a combination of pegylated interferon alpha (PEG-IFNα) and ribavirin (RBV) given for 48 weeks is the standard of care for chronic HCV infection [6, 7]. Predictive factors for achieving a sustained virological response (SVR) have been reported, including pretreatment demographics (age, sex) and clinical [alanine aminotransferase (ALT) level, liver histology], viral (HCV genotype, viral load, amino acid substitutions in the HCV) and treatment (RBV concentration) parameters [2–4, 8, 9].

However, because such a high percentage of patients who complete PEG-IFNα and RBV treatment relapse, defined as having undetectable HCV RNA at the end of treatment but detectable HCV RNA during the follow-up period, it is important to accurately determine the factors involved in relapse. Previous reports have suggested that early viral kinetics, HCV genotype, host (HIV coinfection) and drug (PEG-IFNα and RBV dosing) factors influence the relapse rate [10–12]. From the perspective of drug factors, Shiffman et al. [13], in a study of HCV genotype 1 patients, reported that the relapse rate was lower among patients receiving high-dose (1,000–1,600 mg/day or 15.2 mg/kg/day) compared with standard dose (800–1,400 mg/day or 13.3 mg/kg/day) RBV. Similarly, Fried et al. [14], in a study of HCV genotype 1 patients with high baseline HCV RNA and high body mass index (BMI), reported that a lower relapse rate was attained for patients receiving high doses of PEG-IFNα-2a (270 μg/week) and high doses of RBV (1,600 mg/day) than that attained for patients treated with a standard dosing regimen. These findings indicate that the therapeutic dose, especially that of RBV, plays an important role in reducing relapse in the treatment of chronic HCV infection. However, no medical consensus has been reached regarding evaluation of the treatment dose related to virological relapse (i.e., detectable HCV RNA after HCV RNA has been cleared).

The aim of this large-scale treatment analysis was to assess, in HCV genotype 1 patients treated with PEG-IFNα-2b and RBV for 48 weeks, whether or not the PEG-IFNα and RBV doses had an effect on virological relapse after the clearance of HCV RNA.

Patients and methods

Patients

This prospective study was of 2,871 Japanese patients with chronic HCV infection aged 18 years or older treated with PEG-IFNα-2b and RBV between December 2004 and February 2009. Of the 2,871 patients screened, 1,712 were excluded from the study because of having HCV genotypes other than genotype 1, lack of treatment response evaluation, unclear HCV genotype and viral load, history of hepatocellular carcinoma, or prolonged duration of treatment (more than 48 weeks). Of the remaining 1,159 patients who met the enrollment criteria, 402 were excluded because they did not complete the standard treatment (48 weeks), because of side effects, ineffective virological response, or for economic reasons. To ensure that we were able to accurately evaluate virological relapse, we also excluded patients with a non-viral response (NVR) ($n = 138$). Finally, we investigated the correlation between virological relapse and demographic factors, clinical parameters, and the doses of PEG-IFNα-2b and RBV in 619 patients who had once cleared HCV RNA with PEG-IFNα-2b and RBV treatment. Of these 619 patients, 414 (66.9%) had been treated previously with IFN before being enrolled in this prospective study.

All 619 patients satisfied the following exclusion criteria and were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan : (1) positivity for antibody to human immunodeficiency virus (HIV) or positivity for hepatitis B surface antigen; (2) clinical or biochemical evidence of hepatic decompensation; (3) excessive active alcohol consumption (>60 g/day converted into ethanol) or drug abuse; (4) suspected hepatocellular carcinoma; (5) other forms of liver disease; or (6) treatment with antiviral or immunosuppressive agents prior to enrollment.

Informed consent was obtained from all patients before enrollment. The study was approved by the institutional Ethics Committees of the hospitals involved and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Clinical and laboratory assessments

Clinical parameters assessed included serum albumin, ALT, γ-glutamyl transpeptidase (γGTP), creatinine clearance, hemoglobin, platelet count, plasma glucose, HCV genotype, and HCV RNA. All these parameters were

measured by standard laboratory techniques at a commercial laboratory. Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Insulin resistance was calculated by means of the homeostasis model assessment-insulin resistance (HOMA-IR) method [15].

Therapeutic protocol, dose reduction, and discontinuation of treatment

All patients were treated with PEG-IFNα-2b (PEG-Intron; MSD, Tokyo, Japan) plus RBV (Rebetol; MSD). The duration of treatment for these HCV genotype 1 patients was 48 weeks. PEG-IFNα-2b was given subcutaneously once weekly at a dose of 60–150 μg based on body weight (60 μg for patients weighing 35–45 kg, 80 μg for those weighing 46–60 kg, 100 μg for those weighing 61–75 kg, 120 μg for those weighing 76–90 kg, and 150 μg for those weighing 91–120 kg). RBV was given orally at a daily dose of 600–1,000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60–80 kg, and 1,000 mg for those weighing >80 kg).

Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to <100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFNα-2b-induced psychological adverse effects or a decrease in the white blood cell and platelet counts. In such cases, a reduction in the dose of PEG-IFNα-2b was required. Both PEG-IFNα-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 80 g/L, 1×10^9/L, or 25×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed; continuation of treatment was judged not to be possible by the attending physician; or the patient desired discontinuation of treatment.

Assessment of drug exposure

The doses of PEG-IFNα-2b and RBV were calculated individually as averages on the basis of body weight at baseline by reviewing the patients' medical records: the PEG-IFNα-2b dose was expressed as μg/kg/week and RBV dose was expressed as mg/kg/day.

Determination of HCV-RNA level and HCV genotype

Clinical virological follow up was performed by HCV viremia detection using a real-time reverse transcriptase polymerase chain reaction (PCR) assay (COBAS TaqMan HCV assay; Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 15 IU/mL and an upper limit of quantitation of 6.9×10^7 IU/mL (1.2–7.8 log IU/mL referred to as $\log_{10}$ units/mL). HCV genotype was determined by means of sequence determination in the 5′-nonstructural region of the HCV genome followed by phylogenetic analysis, as previously described [2].

Virological response

The above COBAS TaqMan HCV assay was used to evaluate HCV viremia as a surrogate marker of the virological outcome of treatment. SVR was defined as serum HCV-RNA undetectable at 24 weeks after the end of treatment, and virological HCV relapse was defined as detectable HCV RNA during the 24-week post-treatment period in patients who had undetectable HCV RNA at the end of treatment.

Treatment response was defined as follows: early virological response (EVR), HCV RNA undetectable at week 12; late virological response (LVR), HCV RNA undetectable between week 12 and week 48; and non-viral response (NVR), HCV RNA detectable at the end of treatment.

Liver histology and quantitative variables

Liver biopsy was conducted under ultrasound guidance by experienced hepatologists. For each specimen, the stage of fibrosis and the grade of histological activity were established according to the METAVIR scoring [16]. Fibrosis was staged on a 0–4 scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. The grading of histological activity, including the intensity of necroinflammation, was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

Statistical analysis

Statistical analysis was performed using SAS ver. 9.2 (Statistical Analysis System, SAS Institute, Tokyo, Japan). Quantitative variables were expressed as medians and categorical variables were reported as frequencies and percentages. The paired *t*-test, unpaired *t*-test, Mann–Whitney *U*-test, or χ^2 test was used for the analysis. The significance of trends in values was determined with the Cochran–Armitage trend test. Logistic regression analysis was used to investigate the association of virological response with PEG-IFNα-2b plus RBV treatment, treatment dose, demographic factors, and clinical data. Area under the receiver operating characteristic curve (AUROC) analysis was performed to evaluate the relationship between the RBV dose and virological relapse. The cutoff

values were selected from the receiver operating characteristic (ROC) curve to maximize the total sensitivity and specificity. A two-tailed P value of less than 0.05 was regarded as statistically significant.

Results

Univariate analysis of factors for virological relapse

The overall relapse rate in the present study was 34.1% (211 of 619 patients). The results of univariate analysis of factors for relapse in the patients with virological response are shown in Table 1. The demographic factors of sex (women) and age (65 or over) and the clinical parameters of low creatinine clearance, low albumin, high ALT, low hemoglobin, low platelet count, high HOMA-IR, and liver histology that had progressed were significantly associated with virological relapse. In the multivariate logistic regression analysis, as shown in Table 2, a low total dose of PEG-IFNα-2b and RBV, late timing of HCV RNA negativity, and low average RBV dose were significantly associated with virological relapse. However, the average PEG-IFNα-2b dose and the initial dose of PEG-IFNα-2b or RBV were not associated with relapse.

Relapse rate according to PEG-IFNα-2b and RBV doses (Table 3)

We analyzed the relapse rate according to the degree of exposure to PEG-IFNα-2b and RBV. Table 3 shows the relapse rates according to the PEG-IFNα-2b and RBV doses given over the full treatment period (48 weeks). As a whole, the relapse rate revealed an elevation according to the reduction of RBV dose. The relapse rate for patients who received <6 mg/kg/day of RBV (59.5%, 22 of 37) was significantly higher than the rate for patients in the other RBV dose categories, even if a sufficient dose of PEG-IFNα-2b (≥1.5 μg/kg/day) was received. In contrast, the relapse rate for patients who received ≥12 mg/kg/day of RBV (28.1%, 16 of 57) was significantly lower than the rate for patients in the other RBV dose categories, irrespective of the PEG-IFNα-2b dose.

Multivariate analysis of virological relapse by background and treatment factors (Table 4)

Multivariate analysis was performed to select the background and treatment factors related to virological relapse. For the items affecting virological relapse shown in Table 4, the significant background factors for virological relapse were age (odds ratio 1.68, 95% confidence interval [CI] 1.36–2.08, $P < 0.0001$), ALT (odds ratio 0.93, 95% CI 0.88–0.97, $P = 0.0019$), HOMA-IR (odds ratio 1.24, 95% CI 1.10–1.40, $P = 0.0004$), platelet count (odds ratio 0.95, 95% CI 0.91–0.99, $P = 0.0102$), and liver fibrosis (odds ratio 2.17, 95% CI 1.08–4.34, $P = 0.0290$). Adding the treatment parameters to the analyses, age (odds ratio 1.48, 95% CI 1.20–1.84, $P = 0.0003$), HOMA-IR (odds ratio 1.22, 95% CI 1.08–1.38, $P = 0.0012$), RBV dose (odds ratio 0.90, 95% CI 0.84–0.96, $P = 0.0009$), and LVR (odds ratio 5.75, 95% CI 3.73–8.86, $P < 0.0001$) were found to be independent factors associated with a virological relapse. It is particularly worth noting that the PEG-IFNα-2b dose was not associated with relapse in this multivariate analysis.

Table 1 Baseline characteristics of 619 studied patients with chronic HCV infection

Characteristics	SVR ($n = 408$)	Relapse ($n = 211$)	P value
Men [no. (%)]	224 (54.9)	88 (41.7)	0.0022
Age (years)	55.0 [18–75]	60.0 [26–79]	<0.0001
Body mass index (kg/m^2)	23.1 [14.9–38.0]	23.1 [16.9–37.2]	0.4751
Prior IFN treatment [no. (%)]	277 (67.9)	137 (65.0)	0.3649
Creatinine clearance (L/h)	12.6 [5.1–37.1]	10.6 [4.4–28.6]	0.0002
Albumin (g/dL)	4.2 [3.0–5.1]	4.1 [3.1–4.9]	0.0120
Alanine aminotransferase (IU/L)	61 [14–590]	52 [12–295]	0.0126
γ-Glutamyl-transpeptidase (IU/L)	33 [10–380]	37 [7–255]	0.1225
Hemoglobin (g/L)	139 [96–184]	136 [107–178]	0.0274
Platelet count (10^9/L)	171 [80–343]	154 [61–329]	0.0004
HOMA-IR	1.7 [0.4–33.7]	3.0 [0.4–17.7]	<0.0001
Serum HCV RNA level (log IU/mL)	6.5 [5.0–8.1]	6.6 [5.1–7.6]	0.0888
Liver histology			
Fibrosis: 0–2/3–4	149/101	56/84	0.0002
Activity: 0–1/2–3	102/146	39/98	0.0151

Data are shown as numbers (%) or medians [ranges]

HCV hepatitis C virus, *SVR* sustained virological response, *HOMA-IR* homeostasis model assessment-insulin resistance, *IFN* interferon

Table 2 Treatment doses and virological response of 619 studied patients

Characteristics	SVR ($n = 408$)	Relapse ($n = 211$)	P value
Initial PEG-IFN dose (μg/kg/week)	1.46 [0.54–2.19]	1.45 [0.70–1.98]	0.4063
Initial RBV dose (mg/kg/day)	10.8 [3.4–16.9]	10.6 [3.3–18.0]	0.1065
Average PEG-IFN dose (μg/kg/week)	1.42 [0.54–2.45]	1.37 [0.52–1.94]	0.1506
Average RBV dose (mg/kg/day)	10.0 [3.3–16.6]	9.9 [3.4–13.6]	<0.0001
Assigned total cumulative PEG-IFN dose ≥80% and RBV dose ≥60% [no. (%)]	269 (65.9)	93 (44.1)	<0.0001
Virological response EVR/LVR	363/45	114/97	<0.0001

Data are shown as numbers (%) or medians [ranges]

SVR sustained virological response, *PEG-IFN* pegylated interferon, *RBV* ribavirin, *EVR* early virological response, *LVR* late virological response

Table 3 Relapse rates according to the PEG-IFNα-2b and RBV doses given over the full treatment period (48 weeks)

RBV (mg/kg/day) PEG-IFNα-2b (μg/kg/week)	≥12	10 to <12	8 to <10	6 to <8	<6	Total
≥1.5	19.6% (9/46)	31.8% (14/44)	22.7% (10/44)	35.3% (12/34)	59.5% (22/37)	32.7% (67/205)
1.2 to <1.5	26.3% (5/19)	27.9% (29/104)	30.6% (19/62)	28.9% (13/45)	50.8% (30/59)	33.2% (96/289)
0.9 to <1.2	25.0% (1/4)	17.6% (3/17)	27.8% (5/13)	22.2% (4/18)	58.1% (18/31)	35.2% (31/88)
<0.9	25.0% (1/4)	50.0% (3/6)	16.7% (1/6)	42.9% (3/7)	87.5% (7/8)	48.4% (15/31)
Total	28.1% (16/57)	28.7% (49/171)	26.9% (35/130)	30.8% (32/104)	57.0% (77/135)	34.1% (211/619)

PEG-IFN pegylated interferon, *RBV* ribavirin

ROC curve analysis of association of RBV dose and virological relapse

ROC curve analysis was performed to determine the optimal threshold value of RBV dose for predicting virological relapse. The relevant AUROC was 0.71 and the cutoff value for RBV dose was 6.3 mg/kg/day (sensitivity 85.0%, specificity 44.0%, positive predictive value 74.0%, negative predictive value 62.0%).

Relapse rates by PEG-IFNα-2b dose, sex, age, and the timing of HCV RNA negativity according to the RBV dose (Table 5)

We analyzed the association between the relapse rates and the dose of RBV received with the PEG-IFNα-2b dose divided into ≥1.2 μg/kg/week and <1.2 μg/kg/week. Relapse rates were significantly increased with the reduction of the RBV dose for both PEG-IFNα-2b ≥1.2 and <1.2 μg/kg/week ($P < 0.0001$ and $P = 0.0006$, respectively, by Cochran–Armitage trend test). There was no significant difference between PEG-IFNα-2b ≥1.2 and <1.2 μg/kg/week in any RBV dose category.

The association between relapse rates and the RBV dose received was analyzed by sex. The relapse rates were significantly increased with the reduction of the RBV dose for both men and women ($P < 0.0001$ and $P = 0.0084$, respectively, by Cochran–Armitage trend test). Relapse rates in women were significantly higher than those in men in the group given an average RBV dose of more than 10 mg/kg/day.

The association between relapse rates and the RBV dose received was analyzed by age. The relapse rates were also significantly increased with the reduction of the RBV dose for patients aged 64 or less as well as for those aged 65 or more ($P < 0.0001$ and $P = 0.0037$, respectively, by Cochran–Armitage trend test). The relapse rates of patients aged 65 or more were significantly higher than those of patients aged 64 or less in the group given an average RBV dose of less than 6 mg/kg/day ($P = 0.0069$).

We analyzed the association between relapse rates and the prescribed RBV dose by virological response. The relapse rates were significantly increased with the reduction of the RBV dose both for patients with an EVR and those with an LVR ($P = 0.0006$ and $P = 0.0088$, respectively, by Cochran–Armitage trend test). Relapse rates of patients with an LVR were significantly higher than those of patients with an EVR in the group given an average RBV dose of less than 12 mg/kg/day.

Discussion

Virological HCV relapse is an exceedingly important clinical outcome in the treatment of chronic HCV infection. HCV genotype is a factor well known to affect

Table 4 Multivariate logistic regression analysis of virological relapse by background and treatment factors

Characteristics	Background factors		Background and treatment factors	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age per 10 years	1.68 (1.36–2.08)	<0.0001	1.48 (1.20–1.84)	0.0003
ALT per 10 IU/L	0.93 (0.88–0.97)	0.0019		
HOMA-IR per 1	1.24 (1.10–1.40)	0.0004	1.22 (1.08–1.38)	0.0012
Platelet count per 1 × 10^9/L	0.95 (0.91–0.99)	0.0102		
Fibrosis 3–4 versus 0–2	2.17 (1.08–4.34)	0.0290		
RBV dose per 1 mg/kg/day			0.90 (0.84–0.96)	0.0009
LVR versus EVR			5.75 (3.73–8.86)	<0.0001

ALT alanine aminotransferase, *HOMA-IR* homeostasis model assessment-insulin resistance, *RBV* ribavirin, *LVR* late virological response, *EVR* early virological response, *CI* confidence interval

Table 5 Relapse rates by PEG-IFNα-2b dose, sex, age, and the timing of HCV RNA negativity during treatment according to RBV dose

RBV average dose (mg/kg/day)	RBV ≥12	RBV 10 to <12	RBV 8 to <10	RBV 6 to <8	RBV <6	Cochran–Armitage trend test
PEG-IFNα-2b						
≥1.2 μg/kg/week	21.2 (14/66)	28.7 (43/150)	27.4 (29/106)	32.1 (26/81)	54.2 (52/96)	$P < 0.0001$
<1.2 μg/kg/week	25.0 (2/8)	26.1 (6/23)	25.0 (6/24)	28.0 (7/25)	64.1 (25/39)	$P = 0.0006$
Men	11.1 (4/36)	21.0 (21/100)	24.2 (15/62)	30.6 (15/49)	53.2 (33/62)	$P < 0.0001$
Women	32.4 (12/37)	39.4 (28/71)	29.4 (20/68)	30.9 (17/55)	60.3 (44/73)	$P = 0.0084$
Age						
<65 years	19.4 (12/62)	26.1 (37/142)	25.0 (27/108)	30.1 (22/73)	50.0 (49/98)	$P < 0.0001$
≥65 years	36.4 (4/11)	41.4 (12/29)	36.4 (8/22)	32.3 (10/31)	75.7 (28/37)	$P = 0.0037$
EVR	20.0 (13/65)	16.8 (22/131)	22.2 (24/108)	22.9 (19/83)	41.2 (35/85)	$P = 0.0006$
LVR	37.5 (3/8)	67.5 (27/40)	50.0 (11/22)	61.9 (13/21)	84.0 (42/50)	$P = 0.0088$

Data is shown as the percentile

PEG-IFN pegylated interferon, *HCV* hepatitis C virus, *RBV* ribavirin, *EVR* early virological response, *LVR* late virological response

virological relapse, with HCV genotype 1 patients having a higher relapse rate than HCV genotype 2 or 3 patients (23–30% of HCV genotype 1 patients will experience relapse [11, 17]); however, strategies for the management of relapse are poorly understood. Thus, it is important to research and promote treatment regimens that will insure that patients with the potential to relapse achieve an SVR; such regimens would also improve the cost-effectiveness of treatment and improve the quality of life of patients with chronic HCV infection who are treated with PEG-IFNα and RBV.

The treatment doses used in the present study were strongly associated with virological HCV relapse, with the RBV dose being a more important factor than the PEG-IFNα-2b dose. Our multivariate analyses revealed that while RBV had a dose-dependent correlation with the relapse rate, PEG-IFNα-2b did not. Reducing the dose of RBV to <6 mg/kg/day resulted in an increase in the relapse rate to 57.0% (mean relapse rate was 34.1%) regardless of the PEG-IFNα-2b dose. Shiffman et al. [13] reported that combination treatment of epoetin alpha with PEG-IFNα plus RBV to maintain the hemoglobin level did not enhance SVR or reduce the relapse rate in HCV genotype 1 patients. Thus, maintaining as high an RBV dose as possible throughout the full treatment period may suppress relapse by HCV genotype 1 patients.

Recently, a genome-wide association study identified that polymorphisms of the inosine triphosphatase (ITPA) gene on chromosome 20 influenced RBV-induced anemia [18]. Patients with the ITPA CC genotype at rs1127354 are more likely to develop anemia than those with ITPA CA/AA genotypes during PEG-IFNα and RBV combination treatment for chronic hepatitis C. In fact, Thompson et al. [19] reported that the ITPase deficiency variable (ITPA CA/AA) was associated with a lower rate of anemia-related RBV dose reduction during PEG-IFNα and RBV treatment. Therefore, ITPA genotyping may help guide clinical decisions in considering antiviral treatment.

Although RBV may play an important role in eradicating HCV, RBV does not have a direct antiviral action

against HCV. Immunomodulation exerted by RBV, which acts on CD4 T cells by promoting T-cell differentiation toward the T-helper 1 phenotype, is associated with the clearance of HCV [20], and the proportion of quasi-species defective viruses created due to mutation of the nonstructural 5A and 5B proteins of the HCV genome makes the virus unable to infect new cells [21, 22]. RBV may act synergistically with IFN by upregulating host antiviral proteins or by enhancing IFN signaling [23]. Shiffman et al. [24] suggested that reducing the mean dose of RBV during the first 20 weeks of treatment had little impact on relapse for patients with HCV genotype 1 and they noted that the magnitude of the decline in HCV RNA induced by RBV in the early stages was significantly smaller than that induced by IFN. In the early stages, IFN can induce a decline of several orders of magnitude, while RBV induces a less than 0.5 log decline in HCV RNA. Therefore, the virological effect induced by RBV pharmacokinetics may occur in the later stages.

We confirmed that LVR was a predictive factor for virological relapse, similar to the RBV dose. Based on the results of our study, 37.5% of the patients with an LVR relapsed even when given an average RBV dose of $\geq$12 mg/kg/day. Because it is not necessarily the case that an adequate RBV dose reduces the relapse rate of patients with an LVR, extending the duration of treatment from 48 to 72 weeks must be considered. Relapse rates in HCV genotype 1 slow responders were significantly lower for patients treated with PEG-IFNα-2a and RBV (800 mg/day) for 72 weeks compared with 48 weeks' treatment (40 vs. 64%; $P = 0.021$) [25]. We showed in the present study that the relapse rates of patients with an LVR were significantly higher than those of patients with an EVR for patients treated with an average RBV dose of less than 12 mg/kg/day. However, even for patients with an EVR, the relapse rate increased with a reduction of the RBV dose. Hiramatsu et al. [26] reported a relapse rate of only 4% in HCV genotype 1 patients with an EVR given $\geq$12 mg/kg/day of RBV, and a stepwise reduction of the RBV dose was associated with a stepwise increase in the relapse rate. Therefore, the RBV dose cannot be easily reduced for patients treated with PEG-IFN plus RBV, even if an adequate dose of PEG-IFNα-2b is given, particularly for patients with an LVR.

Treatment of chronic HCV infection will include the addition of direct-acting antivirals with protease inhibitors to PEG-IFNα-2b and RBV regimens. According to some clinical trials, telaprevir, which is an NS3/4A protease inhibitor, has shown promising results, when combined with PEG-IFNα-2b and RBV, in patients infected with HCV genotype 1 [27–29]. However, in telaprevir-based regimens, the addition of RBV increased the SVR rates by preventing relapse and the emergence of telaprevir resistance [29]; therefore, RBV remains a key drug to treat HCV infection.

Host factors, including age, sex, ethnicity, liver histology, and obesity, have been reported to be associated with the outcome of PEG-IFNα and RBV treatment [30–33]. In women and elderly patients who received an adequate dose of RBV, the relapse rates were higher than those in men and younger patients. The mechanisms underlying this are unknown, but indirect evidence (e.g., cellular impairment or impairment of humoral or innate immunity in elderly persons, or a lack of estrogen in elderly women) suggests that chronic infection is associated with phenomena that protect HCV from the antiviral action of IFN and RBV [3, 34, 35].

As mentioned above, the most important result of the present study was that, in patients treated with PEG-IFNα-2b and RBV, relapse after the clearance of HCV RNA was associated with the RBV dose but not with the PEG-IFNα-2b dose. Recently, genetic studies have identified several single nucleotide polymorphisms in and near the interleukin-28B gene region, encoding IFN-λ3, that are correlated with HCV clearance [36–38]. IFN-λ3 upregulates IFN-stimulated genes and affects the adaptive immune response. Fortunately, East Asian populations, including Japanese, have the highest frequencies of the alleles associated with HCV clearance [37], so the PEG-IFNα dose probably did not influence HCV virological relapse to a great degree in our patients.

In conclusion, in HCV genotype 1 patients treated with PEG-IFNα-2b and RBV, the HCV virological relapse rate was dose-dependently correlated with RBV, irrespective of age, sex, the effect of early viral kinetics, or the dose of PEG-IFNα-2b. Careful consideration must be given to decisions on the tapering of the RBV dose, even if an EVR is achieved.

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Original Article

Factors responsible for the discrepancy between *IL28B* polymorphism prediction and the viral response to peginterferon plus ribavirin therapy in Japanese chronic hepatitis C patients

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Aim: *IL28B* polymorphisms serve to predict response to pegylated interferon plus ribavirin therapy (PEG IFN/RBV) in Japanese patients with chronic hepatitis C (CHC) very reliably. However, the prediction by the *IL28B* polymorphism contradicted the virological response to PEG IFN/RBV in some patients. Here, we aimed to investigate the factors responsible for the discrepancy between the *IL28B* polymorphism prediction and virological responses.

Methods: CHC patients with genotype 1b and high viral load were enrolled in this study. In a case–control study, clinical and virological factors were analyzed for 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype who were matched according to sex, age, hemoglobin level and platelet count.

Results: Higher low-density lipoprotein (LDL) cholesterol, lower γ-glutamyltransferase and the percentage of wild-type phenotype at amino acids 70 and 91 were significantly associated with the rs8099917 TT genotype. Multivariate analysis showed that rs8099917 TG or GG genotype, older age and lower LDL cholesterol were independently associated with the non-virological responder (NVR) phenotype. In patients with rs8099917 TT genotype (predicted as virological responder [VR]), multivariate analysis showed that older age was independently associated with NVR. In patients with rs8099917 TG or GG genotype (predicted as NVR), multivariate analysis showed that younger age was independently associated with VR.

Conclusion: Patient age gave rise to the discrepancy between the prediction by *IL28B* polymorphism and the virological responses, suggesting that patients should be treated at a younger age.

Key words: aging, genotype, IL28B, low-density lipoprotein cholesterol, single nucleotide polymorphism

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a global health problem with worldwide estimates of 120–130 million carriers.[1] Chronic HCV infection, the leading cause of liver transplantation, can lead to progressive liver disease, resulting in cirrhosis and complications, including decompensated liver disease and hepatocellular carcinoma.[2] The current standard-of-care treatment for suitable patients with chronic HCV infection consists of pegylated interferon-α-2a or -2b (PEG IFN) given by injection in combination with oral ribavirin (RBV) for 24 or 48 weeks, depending on HCV genotype. Large-scale treatment in the USA and Europe showed that 42–52% of patients with HCV genotype 1

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achieved a sustained virological response (SVR),[3–5] and studies conducted in Japan produced similar results. This treatment is associated with well-known side-effects (e.g. influenza-like syndrome, hematological abnormalities and neuropsychiatric events) resulting in reduced compliance and fewer patients completing treatment.[6] It is important to predict an individual's response before treatment with PEG IFN/RBV to avoid side-effects, as well as to reduce the treatment cost. The HCV genotype, in particular, is used to predict the response: patients with the HCV genotype 2/3 have a relatively high rate of SVR (70–80%) with 24 weeks of treatment, whereas those infected with genotype 1 have a much lower rate of SVR, despite 48 weeks of treatment.[5]

Our recent genome-wide association studies (GWAS) revealed that several highly correlated common single nucleotide polymorphisms (SNP) in the region of the interleukin-28B (*IL28B*) gene on chromosome 19, coding for interferon (IFN)-λ3, are implicated in the non-virological responder (NVR) to PEG IFN/RBV phenotype among patients infected by HCV genotype 1.[7] The association between response to PEG IFN/RBV and SNP associated with *IL28B* was concurrently reported by two other groups who also employed GWAS.[8,9] The *IL28B* polymorphism was highly predictive of the response to PEG IFN/RBV therapy in Japanese chronic hepatitis C (CHC) patients.[10–12] However, this was not always the case. Therefore, we attempted to determine why the *IL28B* polymorphism did not predict the response of all patients. The nature of the functional link between the *IL28B* polymorphism and HCV clearance is unknown, and this must be defined to understand how the *IL28B* polymorphism correlates with HCV clearance. Therefore, we also investigated the association between the *IL28B* polymorphism and clinical characteristics of CHC patients.

METHODS

Patients

A TOTAL OF 696 CHC patients with genotype 1b and high viral load were recruited from the National Center for Global Health and Medicine, Hokkaido University Hospital, Tokyo Medical and Dental University Hospital, Yamanashi University Hospital, Tonami General Hospital, and Shin-Kokura Hospital in Japan. In a case–control study, sex, age, hemoglobin level and platelet count were matched between patients with the rs8099917 TT genotype ($n = 130$) and patients with rs8099917 TG or GG genotypes ($n = 96$) to eliminate background biases.

Each patient was treated with PEG IFN-α-2b (1.5 μg/kg s.c. weekly) or PEG IFN-α-2a (180 μg/body s.c. weekly) plus RBV (600–1000 mg daily, depending on bodyweight). Because a reduction in the dose of PEG IFN/RBV can contribute to a lower SVR rate,[13] only patients with an adherence of more than 80% dose for both drugs during the first 12 weeks were included in this study. Those positive for hepatitis B surface antigen and/or anti-HIV were excluded from this study.

Non-virological response was defined as less than a 2 log-unit decline in the serum level of HCV RNA from the pretreatment baseline value within the first 12 weeks and detectable viremia 24 weeks after treatment. Virological response (VR) was defined as attaining SVR or transient virological response (TVR) in this study; SVR was defined as undetectable HCV RNA in serum 6 months after treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after the treatment was discontinued for a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. At the time of enrollment, written informed consent was obtained for the collection and storage of serum and peripheral blood. This study was conducted in accordance with provisions of the Declaration of Helsinki.

Clinical and laboratory data

The sex, age, hemoglobin (Hb) and platelet counts were matched between study groups. Other parameters determined were as follows: alkaline phosphatase (ALP), alanine transaminase (ALT), total cholesterol, fasting blood sugar (FBS), low-density lipoprotein (LDL) cholesterol, γ-glutamyl transpeptidase (γ-GTP), α-fetoprotein (AFP), HCV RNA level and the rs8099917 polymorphism near *IL28B*.

DNA extraction

Genomic DNA was extracted from the buffy coat fraction of patients' whole blood using a GENOMIX kit (Talent SRL; Trieste, Italy).

IL28B genotyping

We have reported that the rs8099917 polymorphism is the best predictor for the response of Japanese CHC patients to PEG IFN/RBV therapy than other SNP near *IL28B*.[14] Therefore, the rs8099917 polymorphism was genotyped using the InvaderPlus assay (Third Wave Japan, Tokyo, Japan), which combines polymerase

chain reaction (PCR) and the invader reaction.[15,16] The InvaderPlus assay was performed using the LightCycler LC480 (Roche Applied Science, Mannheim, Germany).

Detection of amino acid substitutions in core and NS5A regions of HCV-1b

In the present study, substitutions of amino acid residues 70 (s-aa 70) and 91 (s-aa 91), and the presence of the IFN sensitivity-determining region (ISDR) were determined by direct nucleotide sequencing. HCV RNA was extracted from serum samples at the start of patients' therapy and reverse transcribed with a random primer and SuperScript III reverse transcriptase (Life Technologies, Carlsbad, CA, USA). Nucleic acids were amplified by PCR as described.[17]

Statistical analysis

Quantitative variables were expressed as the mean ± standard error (SE) unless otherwise specified. Categorical variables were compared using a χ^2-test or Fisher's exact test, as appropriate, and continuous variables were compared using the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant. Multivariate analysis was performed using a stepwise logistic regression model. We performed statistical analyses using STATA ver. 11.0 (StataCorp, College Station, TX, USA).

RESULTS

Patient characteristics and *IL28B* genotype in a matched case–control study

TABLE 1 SHOWS PATIENT characteristics according to *IL28B* genotype. In a matched case–control study, sex, age, Hb levels and platelet counts were matched between 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype. Lower γ-GTP ($P = 0.013$) and higher LDL cholesterol levels ($P < 0.001$) were significantly associated with the TT genotype of rs8099917. The percentages of wild type of s-aa 70 and s-aa 91 of patients with the rs8099917 TT genotype were significantly higher than those of patients with rs8099917 TG or GG genotype (s-aa 70: TT vs TG + GG, 68% vs 37% [$P < 0.001$]; s-aa 91: TT vs TG + GG, 68% vs 51% [$P = 0.017$]).

Factors associated with NVR in total patients

Table 2 shows the factors associated with NVR by univariate and multivariate analyses. Univariate analysis showed that older age ($P = 0.002$), lower platelet counts ($P = 0.01$), higher γ-GTP ($P = 0.013$), lower total cholesterol ($P = 0.017$), lower LDL cholesterol ($P < 0.001$) levels and higher AFP levels ($P = 0.019$) were significantly associated with NVR. The percentage of TG or GG genotype of rs8099917 of patients with NVR was

Table 1 Univariate analysis of *IL28B* TT and TG + GG genotypes

Variable	TT genotype ($n = 130$)	TG + GG genotype ($n = 96$)	*P*-value
Sex (% male)	61 (47)	46 (48)	Matched
Age (years), mean (SE)	57.2 (0.8)	57.5 (0.9)	Matched
Hemoglobin (g/dL), mean (SE)	14.3 (0.3)	13.9 (0.2)	Matched
Platelet count (/μL), mean (SE)	16.2 (0.5)	16.0 (0.5)	Matched
ALT (IU/L), mean (SE)	79.4 (5.4)	80.5 (7.8)	0.281
ALP (IU/L), mean (SE)	273.8 (11.7)	283.9 (11.8)	0.313
γ-GTP (IU/L), mean (SE)	63.4 (6.0)	76.0 (6.4)	0.013
Total cholesterol (mg/dL), mean (SE)	177.5 (3.3)	172.3 (3.2)	0.345
LDL cholesterol (mg/dL), mean (SE)	99.0 (2.6)	83.5 (2.8)	<0.001
Fasting blood sugar (mg/dL), mean (SE)	114.1 (4.1)	104.4 (1.9)	0.97
AFP (ng/dL), mean (SE)	9.8 (1.1)	11.5 (1.6)	0.190
HCV RNA (log IU), mean (SE)	6.2 (0.1)	6.1 (0.1)	0.186
s-aa 70 wild type (%)	70/103 (68)	30/81 (37)	<0.001
s-aa 91 wild type (%)	70/103 (68)	41/81 (51)	0.017
ISDR mutation 0–1 point (%)	82/100 (82)	70/81 (86)	0.42

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; SE, standard error.

Table 2 Univariate and multivariate analyses of patients with chronic hepatitis C treated with PEG IFN/RBV with respect to VR and NVR

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 128$)	NVR ($n = 98$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	65 (51)	42 (43)	0.237		
Age (years), mean (SE)	55.6 (0.8)	59.6 (0.9)	0.002	1.075 (1.012–1.143)	0.02
rs8099917 (TG or GG genotype) (%)	23/128 (18)	73/98 (74)	<0.001	25.460 (7.436–87.169)	<0.001
Hemoglobin (g/dL), mean (SE)	14.4 (0.3)	13.7 (0.2)	0.053		
Platelet count (/μL), mean (SE)	16.9 (0.5)	15.0 (0.5)	0.01		
ALT (IU/L), mean (SE)	83.9 (6.4)	74.5 (6.2)	0.116		
ALP (IU/L), mean (SE)	274.1 (12.3)	282.9 (11.2)	0.169		
γ-GTP (IU/L), mean (SE)	65.9 (6.4)	72.6 (5.6)	0.013		
Total cholesterol (mg/dL), mean (SE)	180.3 (3.1)	168.4 (3.5)	0.017		
LDL cholesterol (mg/dL), mean (SE)	100.5 (2.7)	83.5 (2.8)	<0.001	0.978 (0.956–0.999)	0.046
Fasting blood sugar (mg/dL), mean (SE)	106.6 (2.9)	114.8 (4.4)	0.058		
AFP (ng/dL), mean (SE)	9.6 (1.1)	12.0 (1.6)	0.021		
HCV RNA (Log IU), mean (SE)	6.2 (0.1)	6.2 (0.1)	0.876		
s-aa 70 wild type (%)	67/102 (66)	33/82 (54)	0.001		
s-aa 91 wild type (%)	67/102 (66)	44/82 (54)	0.097		
ISDR mutation 0–1 point (%)	79/96 (82)	73/85 (86)	0.511		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; PEG IFN, peginterferon; SE, standard error; RBV, ribavirin; VR, virological response.

significantly higher than that of patients with VR (VR vs NVR: 23/128 [18%] vs 73/98 [74%], $P < 0.001$). The percentage of wild-type s-aa 70 in patients with NVR was significantly lower than that in patients with VR [VR vs NVR: 67/102 [66%] vs 33/82 [54%], $P = 0.001$]. Multivariate analysis showed that older age (odds ratio [OR] = 1.075; 95% confidence interval [CI] = 1.012–1.14; $P = 0.02$), TG or GG genotype of rs8099917 (OR = 25.460; 95% CI = 7.436–87.169; $P < 0.001$) and lower LDL cholesterol levels (OR = 0.978; 95% CI = 0.956–0.999; $P = 0.046$) were independently associated with NVR.

VR to treatment depending on *IL28B* genotype

In the patients with the rs8099917 TT genotype, the rates of SVR, TVR and NVR were 62%, 19% and 19%, respectively. Therefore, 19% patients were NVR, even though rs8099917 represents the TT genotype (predicted as VR). In contrast, in the patients with rs8099917 TG or GG, the rates of SVR, TVR and NVR were 14%, 10% and 76%, respectively. Therefore, 24% patients were VR, even though rs8099917 was TG or GG genotype (predicted as NVR) (Fig. 1).

Factors associated with NVR in patients with the rs8099917 TT genotype

Table 3 shows the factors associated with NVR in patients with the rs8099917 TT genotype (predicted as VR) by univariate and multivariate analyses. Univariate analysis showed that female sex ($P = 0.003$), older age

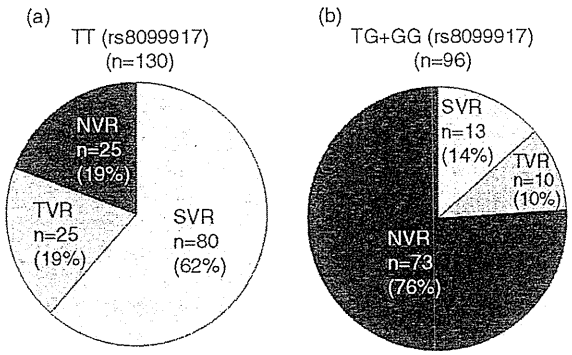

Figure 1 Virological responses to pegylated interferon and ribavirin therapy were shown in patients with rs8099917 TT (a) and TG + GG (b). NVR, non-virological response; SVR, sustained virological response; TVR, transient virological response.

Table 3 Variables associated with NVR by univariate and multivariate analyses in patients with rs8099917 TT genotype

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 105$)	NVR ($n = 25$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	56 (53)	5 (20)	0.003		
Age (years), mean (SE)	56.1 (0.8)	61.7 (1.6)	0.001	1.142 (1.026–1.271)	0.015
Hemoglobin (g/dL), mean (SE)	14.6 (0.4)	13.1 (0.3)	0.005		
Platelet count (/μL), mean (SE)	16.7 (0.6)	13.8 (1.0)	0.019		
ALT (IU/L), mean (SE)	83.6 (6.3)	61.0 (7.9)	0.053		
ALP (IU/L), mean (SE)	270.6 (13.6)	285.9 (22.3)	0.206		
γ-GTP (IU/L), mean (SE)	66.9 (7.1)	49.2 (7.4)	0.473		
Total cholesterol (mg/dL), mean (SE)	180.2 (3.6)	165.0 (7.6)	0.072		
LDL cholesterol (mg/dL), mean (SE)	101.2 (2.9)	88.5 (5.2)	0.067		
Fasting blood sugar (mg/dL), mean (SE)	108.4 (3.5)	140.0 (15.5)	0.127		
AFP (ng/dL), mean (SE)	9.4 (1.2)	12.2 (3.6)	0.245		
HCV RNA (log IU), mean (SE)	6.2 (0.1)	6.2 (0.1)	0.948		
s-aa 70 wild type (%)	57/83 (66)	13/20 (75)	0.752		
s-aa 91 wild type (%)	55/83 (66)	15/20 (75)	0.452		
ISDR mutation 0–1 point (%)	64/79 (81)	18/21 (86)	0.618		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; SE, standard error; VR, virological response.

($P = 0.001$), lower Hb levels ($P = 0.005$) and lower platelet counts ($P = 0.019$) were significantly associated with NVR in patients with the rs8099917 TT genotype. Multivariate analysis showed that only older age was independently associated with NVR in patients with the rs8099917 TT genotype (predicted as VR) (OR = 1.142; 95% CI = 1.026–1.27; $P = 0.015$).

Factors associated with VR in patients with the rs8099917 TG or GG genotypes

Table 4 shows the factors associated with VR in patients with the rs8099917 TG or GG genotypes (predicted as NVR) by univariate and multivariate analyses. Younger age ($P = 0.005$), lower γ-GTP ($P = 0.009$) and higher LDL cholesterol levels ($P = 0.032$) were significantly associated with VR by univariate analysis. Multivariate analysis showed that only younger age was independently associated with VR in patients with the rs8099917 TG or GG genotype (predicted as NVR) (OR = 0.926; 95% CI = 0.867–0.990; $P = 0.023$).

Rate of VR depending on the rs8099917 genotype of each age group

We divided patients into four age groups and compared VR rates by the differences in rs8099917 genotype for each group. The rate of VR decreased gradually in the older age groups independent of genotype. In the less than 49 years age group, the rate of VR in patients with the rs8099917 TT genotype was significantly higher than that in patients with the rs8099917 TG + GG genotypes ($P = 0.0002$). Further, in the 50–59 and 60–69 years age groups, the rates of VR in patients with the rs8099917 TT genotype were significantly higher than those in patients with the rs8099917 TG + GG genotypes ($P < 0.0001$, respectively). In the group that included subjects aged older than 69 years, only 50% of patients achieved VR even in those with the rs8099917 TT genotype (predicted as VR). In contrast, 47.6% of patients achieved VR, including those with the rs8099917 TG or GG genotypes (predicted as NVR) in the less than 49 years group (Fig. 2).

DISCUSSION

SINGLE NUCLEOTIDE POLYMORPHISM array analysis employing GWAS technology conducted by our laboratory and others revealed the relationships between SNP associated with the *IL28B* locus or present within the coding sequences for IFN-λ3, or the response to PEG IFN/RBV therapy for CHC.[7–9] Subsequent studies have confirmed that the response to PEG IFN/RBV therapy correlates with the SNP associated with *IL28B*[18,19] and indicates their value for predicting the response to PEG IFN/RBV therapy. Unfortunately, these predictions do not hold for some patients. In an attempt to understand the reasons for this, in the present study,

Table 4 Variables associated with VR by univariate and multivariate analyses in patients with rs8099917 TG or GG genotypes

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 23$)	NVR ($n = 73$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	9 (40%)	37 (51%)	0.333		
Age (years), mean (SE)	53.2 (1.7)	58.8 (1.1)	0.005	0.926 (0.867–0.990)	0.023
Hemoglobin (g/dL), mean (SE)	13.6 (0.3)	13.9 (0.2)	0.44		
Platelet count (/μL), mean (SE)	17.6 (1.1)	15.5 (0.6)	0.059		
ALT (IU/L), mean (SE)	85.5 (21.6)	78.9 (7.8)	0.767		
ALP (IU/L), mean (SE)	291.9 (28.6)	281.8 (13.0)	0.921		
γ-GTP (IU/L), mean (SE)	62.2 (15.1)	80.4 (6.9)	0.009		
Total cholesterol (mg/dL), mean (SE)	180.5 (6.2)	169.5 (3.7)	0.17		
LDL cholesterol (mg/dL), mean (SE)	97.6 (6.9)	81.9 (3.6)	0.032		
Fasting blood sugar (mg/dL), mean (SE)	98.1 (2.8)	106.3 (2.3)	0.084		
AFP (ng/dL), mean (SE)	10.3 (3.4)	11.9 (1.8)	0.123		
HCV RNA (log IU), mean (SE)	5.9 (0.1)	6.2 (0.1)	0.087		
s-aa 70 wild type (%)	10/19 (53)	20/62 (32)	0.108		
s-aa 91 wild type (%)	12/19 (63)	29/62 (47)	0.211		
ISDR mutation 0–1 point (%)	15/17 (88)	55/64 (86)	0.806		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; SE, standard error; VR, virological response.

we recruited a new set of patients for further analysis. Here, we confirmed that *IL28B* polymorphism was the most significant predictive factor for NVR with respect to PEG IFN/RBV treatment. Moreover, 19% of patients exhibiting the rs8099917 TT genotype were NVR, although they were predicted as VR. Twenty-four percent of patients with the rs8099917 TG or GG genotypes were VR, although they were predicted as NVR. We were able to determine by multivariate analysis that age was the most likely factor responsible for the discordance

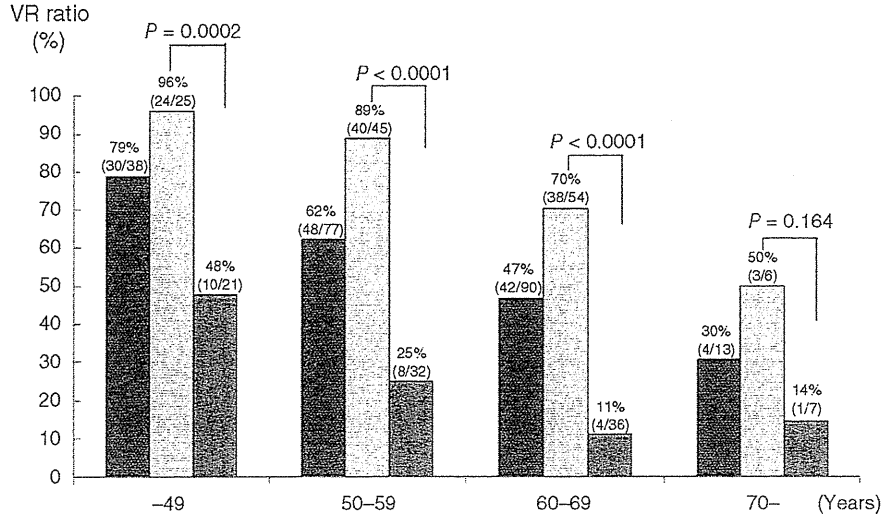

Figure 2 Virological responses (VR) to pegylated interferon and ribavirin therapy were compared between the patients with rs8099917 TT and TG + GG in each generation group. (■) Total patients, (□) TT genotype (rs8099917), (▤) TG + GG genotype (rs8099917).

between *IL28B* genotype and patients' response to viral infection.

How does age influence the VR to PEG IFN/RBV therapy? First, the lower rate of VR to PEG IFN/RBV therapy in patients with CHC was attributed to lower compliance with the IFN or RBV dose.[20,21] Because lower compliance with PEG IFN or RBV therapy was expected to be associated with a lower rate of VR in older patients, we recruited patients who were administrated over 80% of the prescribed dose of IFN/RBV. Therefore, lower compliance can be discounted as a reason for reduced response. Second, a more advanced stage of fibrosis might have been present in the older group. Platelet counts in patients with NVR were significantly lower than those in patients with VR, and lower platelet counts may be associated with advanced fibrosis.[22] Moreover, advanced fibrosis is associated with lower rates of SVR to IFN-based therapy.[23] Third, epigenetic factors such as DNA methylation induced by aging may be involved in the reduced efficacy of PEG IFN/RBV treatment in older patients. DNA methylation near gene promoters is known to turn off transcription or reduce it considerably,[24] and advanced age is strongly associated with the increased DNA methylation.[25] Therefore, DNA methylation may be increased near or in the *IL28B* promoter as a function of age resulting in suppression of *IL28B* transcription.

Lower LDL cholesterol levels were significantly associated with NVR in patients with CHC. Moreover, LDL cholesterol levels in patients with the rs8099917 TT genotype were significantly higher than those in patients with the TG + GG genotypes. The association between LDL cholesterol and *IL28B* polymorphism as well as the VR to PEG IFN/RBV has been reported.[26] Higher pretreatment levels of LDL cholesterol have been shown to predict increased response to standard PEG IFN/RBV treatment for patients with CHC.[27,28] Although the mechanisms responsible for the association between LDL cholesterol levels and the VR to PEG IFN/RBV are unknown, the *IL28B*-rs8099917 TT responder genotype, which may correlate with an increased likelihood of treatment response and higher LDL cholesterol levels, is associated with either lower IFN-λ3 activity or reduced expression of genes regulated by IFN-mediated signaling pathways.

In conclusion, our studies provide compelling evidence that patient age is most likely responsible for incorrect predictions of VR to PEG IFN/RBV therapy in Japanese CHC patients based on *IL28B* genotypes. Our findings indicated that patients should be treated as soon as they are diagnosed. It will be important to investigate the role of the epigenetic factors associated with *IL28B* expression to develop more effective PEG IFN/RBV-based therapies for patients with CHC.

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HEPATOLOGY

Evaluation of the adverse effect of premature discontinuation of pegylated interferon α-2b and ribavirin treatment for chronic hepatitis C virus infection: Results from Kyushu University Liver Disease Study

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Conflict of interest
None declared.

Abstract

Background and Aims: Pegylated interferon (PEG-IFN) α-2b and ribavirin (RBV) treatment of chronic hepatitis C virus (HCV) infection is associated with a substantially elevated risk of discontinuation. The aim of this study is to evaluate the reason for premature discontinuation during PEG-IFN α-2b and RBV treatment due to adverse effects in patients with chronic HCV infection.

Methods: A total of 2871 Japanese patients who had chronic HCV infection treated with PEG-IFN α-2b and RBV were screened. We prospectively investigated the reasons for premature discontinuation of treatment classified by sex and age, and analyzed the timing of discontinuation.

Results: Of the 2871 patients, 250 (8.7%) discontinued treatment because of adverse effects. The main reasons for premature discontinuation were neurovegetative symptoms ($n = 77$, 30.8%), depression-related syndrome ($n = 46$, 18.4%), hematologic effects ($n = 41$, 16.4%) and dermatologic effects ($n = 27$, 10.8%). The rate of discontinuation of treatment for patients aged ≥ 65 years was significantly higher than for patients aged < 65 years, for both men ($P < 0.0001$) and women ($P = 0.0121$). Moreover, the frequency of discontinuation due to neurovegetative symptoms, depression-related syndrome, and hematologic effects for men aged ≥ 65 years was significantly higher than for those aged < 65 years ($P = 0.0001$, $P = 0.0016$, and $P = 0.0170$, respectively), but not for women.

Conclusion: Premature discontinuation due to the adverse effects of PEG-IFN α-2b and RBV treatment by patients with chronic HCV infection is mainly due to neuropsychiatric symptoms and is more common for older than for younger patients.

Introduction

Hepatitis C virus (HCV) infection is the main cause of chronic liver disease and is reported to have infected approximately 170 million people worldwide. Most patients with chronic HCV infection may achieve normal life expectancy, but about 30% develop the life-threatening complications of end-stage liver disease, including cirrhosis and hepatocellular carcinoma.[1,2] Antiviral treatment with interferon (IFN) for chronic HCV infection can induce viral clearance and biochemical and histological improvement.[3–5]

Although antiviral treatment has steadily improved over the last decades, the rates of sustained virological response (SVR) were only 40–50% for patients treated with pegylated IFN (PEG-IFN) α and ribavirin (RBV) for chronic hepatitis infected with HCV genotype 1.[6,7] SVR depends mainly on factors such as viral (HCV genotype, the HCV core 70[8] and NS5A interferon sensitivity-determining regions[9]), host factors (polymorphisms in the

interleukin 28B region[8,9]), and the early viral kinetics of antiviral treatment.[10,11] One of the reasons for the low SVR rates is the high frequency of adverse events related to PEG-IFN α and RBV treatment;[12] therefore, the adherence to antiviral treatment would have a favorable effect on the SVR rate. In fact, patients who discontinued PEG-IFN α and RBV treatment prematurely for whatever reason had an SVR rate of 12% compared with 65% of those who continued treatment despite dose reduction.[13]

Laboratory abnormalities, such as neutropenia, anemia and thrombocytopenia, are the most frequent side-effects of PEG-IFN α and RBV treatment. Dose reductions of either PEG-IFN α and/or RBV for mainly laboratory abnormalities were required by 32–42% of patients in one study; however, the rate of premature discontinuation due to laboratory abnormalities was only 2–3%.[3,7] Although the most common adverse events of PEG-IFN α and RBV treatment were fatigue (64%), headache (62%) and injection-site reaction (58%) in another study,[14] there have been few large-scale reports of the correlation between adverse effects and the premature discontinuation of PEG-IFN α and RBV treatment.

The aim of this large-scale, prospective study was to assess the reasons for and frequency of premature discontinuation of PEG-IFN α-2b and RBV treatment of Japanese patients with chronic HCV infection due to adverse effects.

Methods

Patients. This prospective study was of 2871 Japanese patients with chronic HCV infection aged 18 years or older treated with PEG-IFN α-2b and RBV between December 2004 and February 2009. The number with HCV genotype 1 was 2018 (70.3%, median age: 59 years), of whom 1066 (52.8%) were men. The number with HCV genotype 2 was 853 (29.7%, median age: 54 years), of whom 430 (50.4%) were men. Exclusion criteria were as follows: (i) positivity for antibody to HIV or positivity for hepatitis B surface antigen; (ii) clinical or biochemical evidence of hepatic decompensation; (iii) excessive active alcohol consumption (> 60 g/day converted into ethanol) or drug abuse; (iv) suspected hepatocellular carcinoma at entry; (v) other forms of liver disease (e.g. autoimmune hepatitis, alcoholic liver disease, hemochromatosis); and (vi) treatment with antiviral or immunosuppressive agents prior to enrollment. A total of 2871 patients were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of each participating hospital. Informed consent was obtained from all patients before enrollment.

Clinical and laboratory assessment. Clinical parameters included serum albumin, alanine aminotransferase (ALT), γ-glutamyl-transpeptidase, creatinine clearance (Ccr), total cholesterol, hemoglobin (Hb), complete platelet counts, HCV genotype and HCV RNA. All were measured by standard laboratory techniques at a commercial laboratory. Body mass index (BMI) was calculated as weight in kilograms/height in square meters.

Determination of HCV-RNA level and HCV genotype. Clinical virological follow up was performed by HCV viremia detection using a real-time reverse transcriptase polymerase chain reaction assay (COBAS TaqMan HCV assay; Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9×10^7 IU/mL (1.2–7.8 log IU/mL referred to $\log_{10}$ units/mL). HCV genotype determination was performed by means of sequence determination in the 5′-nonstructual (NS) region of the HCV genome followed by phylogenetic analysis, as previously described.[15]

Therapeutic protocol. All patients received a combination treatment of PEG-IFN α-2b (PEG-Intron; MSD, Tokyo, Japan) plus RBV (Rebetol: MSD). The length of treatment was 48 weeks for HCV genotype 1 and 24 weeks for HCV genotype 2. PEG-IFN α-2b was administered subcutaneously once weekly at a dose of 60–150 μg based on bodyweight (60 μg for patients weighing 35–45 kg, 80 μg for those weighing 46–60 kg, 100 μg for those weighing 61–75 kg, 120 μg for those weighing 76–90 kg and 150 μg for those weighing 91–120 kg). RBV was given orally at a daily dose of 600–1000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing > 80 kg). The above dosages and duration are those approved by the Japanese Ministry of Health, Labor and Welfare.

In the event of a serious adverse effect developing during the course of treatment, we modified the dosage of PEG-IFN α-2b and RBV until the adverse event abated or decreased in severity. Patients were considered to have RBV-induced anemia if the Hb level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFN α-2b-induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of PEG-IFN α-2b was required.

Discontinuation of PEG-IFN α-2b and RBV treatment. Both PEG-IFN α-2b and RBV were discontinued if the Hb level, white blood cell count, or platelet count fell below 85 g/L, 1×10^9 /L, or 25×10^9 /L, respectively. The treatment was discontinued if severe general fatigue, severe neuropsychiatric symptoms, uncontrolled thyroid disease, interstitial pneumonia, progressive IFN retinopathy, the onset of carcinoma, severe hematologic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment.

Virological response. COBAS TaqMan HCV assay was used to evaluate HCV viremia as a surrogate marker of virological outcome to treatment. SVR was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment and virological HCV relapse was defined as detectable HCV RNA during the 24-week post-treatment period of patients who had undetectable HCV RNA at the end of treatment.

Definition of neuropsychiatric symptoms and the assessment of psychiatric problems. Neuropsychiatric symptoms included two distinct dimensions; a neurovegetative symptom and a depression-related syndrome.[16] The neurovegetative symptoms were reduced energy, anorexia, and psychomotor retardation, while depression-related syndromes were worsening

mood, anxiety, suicidal ideation, and aggressive behavior towards others. All patients were seen by hepatologists and psychiatrists at least weekly but as often as necessary during the first 8 weeks and then once a month. Mental status was continuously, carefully monitored. Major depressive episodes during PEG-IFN α-2b and RBV treatment were diagnosed by clinical assessment according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.[17]

Statistical analysis. Statistical analysis was performed using SAS ver. 9.2 (SAS Institute, Cary, NC, USA). Quantitative variables were expressed as median and interquartile range. The paired *t*-test, unpaired *t*-test, Mann–Whitney *U*-test, χ^2-test or the Kruskal–Wallis test were used where appropriate. A *P*-value less than 0.05 was regarded as statistically significant.

Results

Rates of premature discontinuation of PEG-IFN α-2b and RBV treatment (Table 1). Of the 2871 patients screened, 551 (19.2%) had PEG-IFN α-2b and RBV treatment discontinued during the treatment period. The discontinuation rate of patients with HCV genotype 1 (209 of 2010, 10.4%) due to adverse effects was more than double that of those with HCV genotype 2 (41 of 861, 4.8%) ($P < 0.0001$), possibly due to the difference of the treatment period. Of the 551, 250 (45.4%) had treatment discontinued because of adverse effects and 301 (54.6%) discontinued for other reasons (e.g. non-virological response, the onset of carcinoma, economic reasons, or dropout).

The rate of premature discontinuation of treatment due to adverse effects was significantly higher for patients aged ≥ 65 years than for those aged < 65 years, both for men (14.4% *vs* 7.3%, $P < 0.0001$) and women (11.2% *vs* 7.0%, $P = 0.0121$). Although the rates of premature discontinuation of treatment were not significantly different for patients aged ≥ 65 years and those < 65 years with HCV genotype 2, for both men and women, the discontinuation rates of the group of patients aged ≥ 65 years with HCV genotype 1 was significantly higher than that of the group aged < 65 years, both for men (16.9% *vs* 8.3%, $P < 0.0001$) and women (13.6% *vs* 8.4%, $P = 0.0143$).

Demographic characteristics of the studied 250 patients who discontinued PEG-IFN α-2b and RBV treatment prematurely (Table 2). Demographic characteristics of the 209 patients with HCV genotype 1 and the 41 with HCV genotype 2 who discontinued the combined treatment are compared in Table 2. They all discontinued PEG-IFN α-2b and RBV treatment prematurely because of adverse effects during the treatment period. The age was significantly higher ($P = 0.0002$) and Ccr and platelet count were lower ($P = 0.0003$ and $P = 0.0139$, respectively) for patients with HCV genotype 1 than for those with genotype 2. This was due to the age difference between HCV genotype 1 (median 59 years) and 2 (median 54 years) ($P < 0.0001$) patients at entry to the study.

Breakdown of the reasons for premature discontinuation of PEG-IFN α-2b and RBV treatment (Table 3). The reasons for premature discontinuation of PEG-IFN α-2b and RBV treatment included neurovegetative symptoms ($n = 77$, 30.8%), depression-related syndrome ($n = 46$, 18.4%), hematologic effects ($n = 41$, 16.4%), dermatologic effects ($n = 27$, 10.8%), thyroid disease ($n = 10$, 4.0%), pulmonary disease (including interstitial pneumonia and tuberculosis) ($n = 10$, 4.0%), IFN-induced retinopathy ($n = 6$, 2.4%), autoimmune disease ($n = 4$, 1.6%), elevation of ALT (over 10 times the upper limit of the normal range) ($n = 2$, 0.8%), cerebral vascular disease ($n = 2$, 0.8%), diabetes mellitus type 1 ($n = 2$, 0.8%) and others ($n = 23$, 9.2%).

For HCV genotype 1 ($n = 209$), the reasons were neurovegetative symptoms ($n = 69$, 33.0%), depression-related syndrome ($n = 38$, 18.2%), hematologic effects ($n = 33$, 15.8%), dermatologic effects ($n = 20$, 9.6%), thyroid disease ($n = 8$, 3.8%), pulmonary disease (including interstitial pneumonia and tuberculosis) ($n = 8$, 3.8%), IFN-induced retinopathy ($n = 5$, 2.4%), autoimmune disease ($n = 3$, 1.2%), cerebral vascular disease ($n = 2$, 1.0%), diabetes mellitus type 1 ($n = 2$, 1.0%), elevation of ALT ($n = 1$, 0.5%) and others ($n = 20$, 9.6%). Similarly, for HCV genotype 2 ($n = 41$), the reasons were neurovegetative symptoms ($n = 8$, 19.5%), depression-related syndrome ($n = 8$, 19.5%), hematologic effects ($n = 8$, 19.5%), dermatologic effects ($n = 7$, 17.1%), thyroid disease ($n = 2$, 4.9%), interstitial pneumonia

Table 1 Rates of premature discontinuation due to AE of PEG-IFN α-2b and RBV treatment

	Overall			HCV Genotype 1			HCV Genotype 2		
	Total	Discontinuation due to AE	*P*-value*	Total	Discontinuation due to AE	*P*-value*	Total	Discontinuation due to AE	*P*-value*
All patients	2871	250 (8.7)		2010	209 (10.4)		861	41 (4.8)	
Men									
< 65 years	1104	81 (7.3)	< 0.0001	756	63 (8.3)	< 0.0001	348	18 (5.2)	0.7946
≥ 65 years	397	57 (14.4)		308	52 (16.9)		89	5 (5.6)	
Women									
< 65 years	995	70 (7.0)	0.0121	667	56 (8.4)	0.0143	328	14 (4.3)	> 0.9999
≥ 65 years	375	42 (11.2)		279	38 (13.6)		96	4 (4.2)	

Data is shown as *n* (%).

*Comparison of patients aged < 65 years and ≥ 65 years.

AE, adverse effects; HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin.

Table 2 Baseline characteristics of 250 patients with chronic HCV infection who discontinued PEG-IFN α-2b and RBV treatment prematurely due to adverse effects

Characteristics	Total	HCV		*P*-value*
		Genotype 1	Genotype 2	
	n = 250	*n* = 209	*n* = 41	
Men, *n* (%)	138 (55.2)	116 (55.6)	22 (53.7)	0.2428
Age (years)	62 [16]	63 [15]	56 [25]	0.0002
Body mass index (kg/m^2)	23.2 [4.0]	23.1 [3.8]	23.2 [5.3]	0.2428
Creatinine clearance (mL/min)	86 [34]	84 [31]	104 [45]	0.0003
Albumin (g/L)	40 [6]	40 [6]	41 [8]	0.3663
ALT (IU/L)	57.0 [60.0]	61.0 [56.5]	48.5 [79.7]	0.3354
γ-GT (IU/L)	48.0 [56.3]	48.0 [57.0]	45.0 [54.0]	0.4948
Total cholesterol (mg/dL)	166 [41]	162 [41]	175 [35]	0.4322
White blood cells (10^9/L)	47 [18]	47 [18]	50 [26]	0.4145
Hemoglobin (g/L)	137 [21]	136 [21]	141 [24]	0.1333
Platelets (10^9/L)	144 [81]	141 [70]	187 [94]	0.0139
HCV RNA (log IU/mL)	6.5 [1.0]	6.5 [0.9]	5.9 [1.6]	0.1507

Data is shown median [interquartile range] or *n* (%).
*Compared with HCV genotype 1 and 2.
ALT, alanine aminotransferase; γ-GT, γ-glutamyl-transpeptidase; HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin.

Table 3 Breakdown of reasons for premature discontinuation due to adverse effects of PEG-IFN α-2b and RBV treatment

	Genotype 1 (*n* = 209)	Genotype 2 (*n* = 41)	Total (*n* = 250)
Neurovegetative symptom, *n*	69	8	77
Depression-related syndrome, *n*	38	8	46
Hematologic effect, *n*	33	8	41
Anemia	18	3	21
Thrombocytopenia	8	3	11
Neutropenia	7	2	9
Dermatologic effect, *n*	20	7	27
Thyroid disease, *n*	8	2	10
Hyperthyroidism	7	1	8
Hypothyroidism	1	1	2
Pulmonary disease, *n*	8	2	10
Interstitial pneumonia	6	2	8
Pulmonary tuberculosis	2	0	2
Retinopathy, *n*	5	1	6
Autoimmune disease, *n*	3	1	4
Rheumatoid arthritis	2	1	3
Myasthenia gravis	1	0	1
Elevation of ALT†, *n*	1	1	2
Cerebrovascular disease, *n*	2	0	2
Diabetes mellitus type 1, *n*	2	0	2
Others, *n*	20	3	23

†Over 10 times the upper limit of the normal range.
ALT, alanine aminotransferase; PEG-IFN, pegylated interferon; RBV, ribavirin.

(*n* = 2, 4.9%), IFN-induced retinopathy (*n* = 1, 2.4%), autoimmune disease (*n* = 1, 2.4%), elevation of ALT (*n* = 1, 2.4%), and others (*n* = 3, 7.3%).

About half of the premature discontinuations by both genotype 1 and 2 patients were for neuropsychiatric symptoms (123 of 250, 49.2%).

Timing of discontinuation of PEG-IFN α-2b and RBV treatment classified by the type of adverse effect (Fig. 1a,b). In the case of premature discontinuation due to neuropsychiatric symptoms, including neurovegetative symptoms and depression-related syndrome, 66 of 107 patients (61.7%) with HCV genotype 1 discontinued between 5 and 24 weeks after the start of treatment. For premature discontinuation due to hematologic effects, 32 of 33 patients (97.0%) with HCV genotype 1 discontinued within the first 36 weeks of treatment. For premature discontinuation due to dermatologic effects, 11 of 20 patients (55.0%) with HCV genotype 1 discontinued within the first 12 weeks of treatment. However, analyses of HCV genotype 2 found no significant differences in the timing of discontinuation of treatment among the tested adverse effects.

Breakdown of the reasons for premature discontinuation classified by sex, age, and adverse effects (Table 4). Of the 250 patients who prematurely discontinued treatment, 138 were men and 112 women ($P = 0.3336$). The most common reason for premature discontinuation was neuropsychiatric symptoms (neurovegetative symptoms and depression-related syndrome), both for men (64 of 138, 46.3%) and women (59 of 112, 52.7%). No significant differences in premature discontinuation due to neurovegetative symptoms, depression-related syndrome, or dermatologic effects were found in the sex-based analysis, except for a difference in premature discontinuation due to hematologic effects (men 2.1% *vs* women 0.7%, $P = 0.0026$).

In analyses of neurovegetative symptoms, depression-related syndrome, and hematologic effects classified by sex, age, and adverse effects, the rate of discontinuation of treatment for male patients aged ≥ 65 years was significantly higher than for male patients aged < 65 years ($P = 0.0001$, $P = 0.0016$ and $P = 0.0170$, respectively); however, no such difference was found for women.