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Original Article

Predicting the response to 48-week combination therapy with peginterferon α-2b plus ribavirin from the estimated HCV RNA load index after negative serum change in genotype 1b hepatitis C patients

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Aim: We estimated viral dynamics after serum hepatitis C virus (HCV) RNA became negative and assessed the relation between the estimated viral load at the end of treatment (EVE) index and the response to the combination therapy with peginterferon α-2b plus ribavirin.

Methods: Patients with chronic HCV, genotype 1b, and a high viral load were treated with this combination therapy for 48 weeks, and serum HCV RNA was measured frequently during the treatment period. In the patients showing an end-of-treatment response (ETR), the viral load profile from the start of treatment until serum HCV RNA became negative was expressed by an approximate curve. Then the EVE index was calculated by using the expression obtained from the curve, and differences between the sustained virologic response (SVR) and relapse groups were investigated. Results: The SVR rate increased as the EVE index became lower, and the EVE index was significantly lower in the SVR group than in the relapse group. The SVR rate was higher for those in whom the EVE index was below the cut-off point.

Conclusion: Prediction of SVR and relapse from the EVE index is more useful than prediction from viral dynamics at the time when HCV RNA becomes negative or when HCV RNA shows a decrease of 2-log or more.

Key words: hepatitis C virus, peginterferon, virologic response

INTRODUCTION

COMBINATION THERAPY WITH peginterferon (Peg-IFN) plus ribavirin (RBV) is a standard treatment for chronic hepatitis C around the world. However, the virologic efficacy of this combination is lower for chronic hepatitis C virus (HCV) patients with genotype 1 and a high viral load than for other patients. The sustained virologic response (SVR) rate after 48 weeks of treatment at a standard dose is approximately 40–50%, which is not satisfactory.[1–4] The virologic response soon after the start of treatment is classified as no response (NR: HCV RNA is detected throughout the treatment period), rapid virologic response (RVR: HCV RNA is not detected after 4 weeks of treatment), complete early virologic response (cEVR: not an RVR, but HCV RNA is not detected after 12 weeks of treatment), partial virologic response (pEVR: HCV RNA is detected after 12 weeks of treatment, but the load is reduced by 2 logs or more compared with before treatment), and late virologic response (LVR: HCV RNA is not detected after 12 weeks or more of treatment).

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According to previous reports, treatment was discontinued early in patients in whom SVR could not be expected with interferon therapy,[5,6] while an appropriate treatment period was selected so that relapse was decreased and the SVR rate was increased when interferon therapy was promising.[6–12] It has also been reported that the SVR rate was higher at 72 weeks than at 48 weeks after starting treatment in patients in whom RVR was not noted at the time when HCV RNA became negative.[7,11,12]

According to the 2007 Japanese guidelines, it is recommended that combined therapy with Peg-IFN plus RBV for chronic HCV with genotype 1 and a high viral load should be continued for 48 weeks in the case of cEVR and for 72 weeks in the case of LVR. It has also been reported that a 24-week treatment period may possibly be appropriate for the RVR group, in whom HCV RNA becomes negative within 4 weeks of starting treatment.[13,14] Since the optimum treatment period differs for each patient, it is useful for improving patient motivation to clarify the SVR rate at the end of each treatment period and also the treatment period that is sufficient to attain SVR in each patient. However, it is actually impossible to measure the residual viral load and the extent of its decrease, especially in the liver, when serum HCV RNA becomes undetectable soon after the start of interferon therapy. If there is an index that estimates the viral load at the end of treatment (EVE index), which is calculated from the changes of viral load between the start of therapy and the time when serum HCV RNA becomes undetectable, it would become possible to predict the SVR rate during shorter treatment periods and select the optimum treatment period to prevent relapse for each patient. However, such estimation has not been attempted on the basis of viral dynamics after serum HCV RNA becomes undetectable.

The objective of the present study was to investigate whether SVR and relapse could be predicted by the EVE index.

MATERIALS AND METHODS

Patients

COMBINATION THERAPY WITH Peg-IFNα2b plus RBV was performed in 106 patients with chronic HCV who were enrolled by the Hiroshima Liver Disease Study Group from January to December 2005. All of them met the following criteria: greater than 18 years of age, a persistent increase of serum alanine transaminase for the past 6 months, positive for anti-HCV antibody by a third-generation enzyme immunoassay (Chiron, Emerville, CA), and HCV genotype 1b. Each patient also had a high viral load ($\geq 1.0 \times 10^5$ IU/mL) on quantitative analysis of HCV RNA by PCR with Cobas Amplicore HCV monitor, version 2.0, using the 10-fold dilution method (Roche Diagostic Systems, Tokyo) at the start of treatment, and histologic evidence of chronic hepatitis on examination of a liver biopsy specimen obtained within the previous 24 months. Exclusion criteria included decompensation of liver function, co-existing serious medical or psychiatric illness, liver disease other than that caused by HCV infection, a neutrophil count less than 1.5×10^9/L, a platelet count less than 80×10^9/L, a hemoglobin less than 12 g/dL, a serum creatinine greater than 1.5 times the upper limit of the normal range, and co-infection with hepatitis A virus, hepatitis B virus, or human immunodeficiency virus. Patients were also excluded if they had received any systemic antiviral, antineoplastic, or immunomodulatory therapy within 6 months before the study. Pregnant and breast-feeding women and male partners of pregnant women were also excluded. All participants had to use two forms of effective contraception during treatment and throughout the 24-week follow-up phase of the study.

Study design

All patients were treated with Peg-IFNα2b (1.5 μg/kg subcutaneously) once weekly for a 48-week period, plus RBV at a dose adjusted for body weight (patients over 80 kg in weight received 1000 mg, those weighing from 60–80 kg received 800 mg, and those under 60 kg received 600 mg). A post-treatment follow-up period of 24 weeks was also included in the study.

HCV RNA detection and viral kinetic studies

Serum samples were frozen at −80°C within 4 h of collection and then were thawed at the time of measurement. The HCV genotype was determined by the polymerase chain reaction (PCR) using a mixed primer set based on the nucleotide sequence of the NS5 region.[15] The serum HCV RNA level was measured with a quantitative HCV RNA assay (Cobas Amplicore HCV monitor ver 2.0; Roche Diagostic Systems, Tokyo, Japan) using the 10-fold dilution method before, during, and after therapy. The range of the assay was 5.0×10^3 to 5.0×10^6 IU/mL. When the measured

serum HCV RNA level was 5.0×10^3 IU/mL, HCV RNA was also determined by a quantitative PCR assay (Amplicor HCV v2.0®, Roche Diagnostic Systems, Tokyo, Japan), which had a detection limit of 50 IU/mL. The response of the patients was classified as follows: end-of-treatment response (ETR: quantitative HCV RNA assay showed that HCV RNA was below the detection limit of 50 IU/mL at the end of treatment), SVR (quantitative HCV RNA assay showed that serum HCV RNA was still below the detection limit at 24 weeks after the end of treatment), relapse (serum HCV RNA was below the detection limit at the end of treatment, but was detected again by 24 weeks after the end of treatment), and no response (NR) (serum HCV RNA was not below the detection limit at the end of treatment). Routine laboratory tests, including HCV RNA determinations, were performed at each participating institution.

Viral dynamics were assessed by measuring the serum HCV RNA level before the initial dose (0 W), after 2 and 4 weeks of treatment, and then at least every 4 weeks during the remainder of the study period. When ETR was noted, the viral load until serum HCV RNA became negative was expressed in units of log IU/mL, and an approximate exponential equation was generated from the weekly changes by using Microsoft Office Excel 2003 for Windows (Microsoft Corporation, Washington, DC; Fig. 1a). Then, assuming that the HCV RNA load continued to decrease in a similar manner after it became negative, the load after 48 weeks of treatment was estimated by extrapolation with this equation (Fig. 1b). The estimated viral load at the end of treatment was defined as the EVE value. When the viral load profile shown in Figure 1a was plotted as logarithmic (log) values on the ordinate for simplicity, the EVE value was 4.6×10^{-3} (Fig. 1c). Differences between the SVR group and the relapse group were investigated. When calculations were done, all HCV RNA levels exceeding 5.0×10^6 IU/mL were regarded as equal to 5.0×10^6 IU/mL (6.7 log IU/mL) for convenience, while HCV RNA levels under 5.0×10^3 IU/mL were regarded as equal to 5.0×10^3 IU/mL (3.7 log IU/mL) when HCV RNA was first found to be below 5.0×10^3 IU/mL. Also, the HCV RNA level was regarded as being equal to 50 IU/mL (1.7 log IU/mL) when a level below the detection limit was first noted. Liver biopsy specimens were examined by specialist pathologists, who assigned a numerical score for necroinflammatory activity (Grades 0 to 3) and another score for fibrosis and architectural distortion (stages 0 to 4) according to the method of Desmet *et al.*[16]

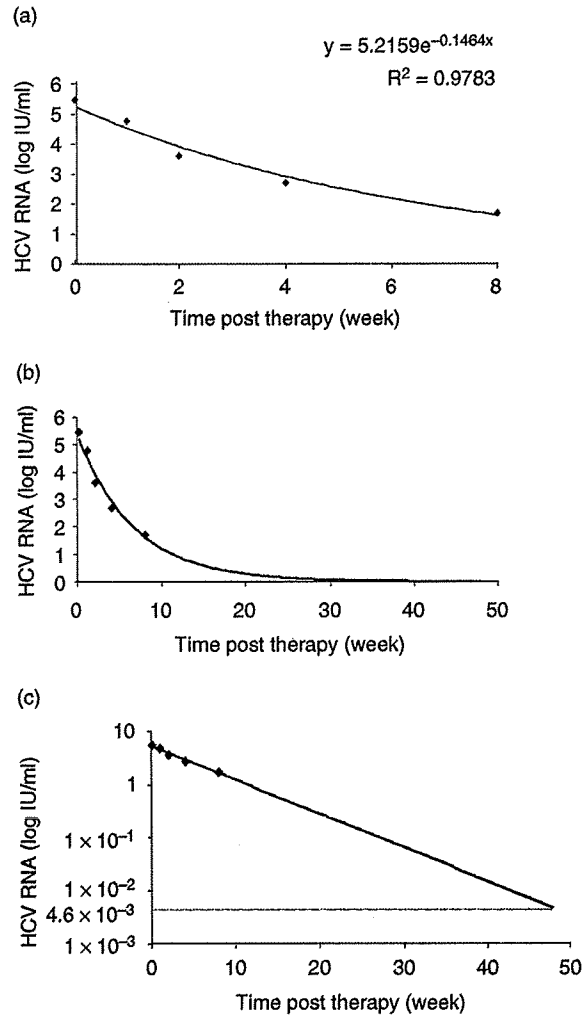

Figure 1 (a) The curve drawn from the equation shows the profile hepatitis C virus (HCV) RNA load. (b) Viral load profile estimated extrapolation for 48 weeks from the curve shown in Figure 1a. (c) The EVE index profile plotted on the ordinate in Figure 1b is express as log values.

Statistical analysis

Differences between groups were examined for statistical significance by Wilcoxon's test and the χ^2-test as appropriate. A P value of less than 0.05 (two-tailed) was considered significant. Statistical analysis was performed with JMP software (JMP USA, Cary, NC). The sensitivity, specificity, positive predictive value, and negative predictive value were also calculated. Receiver operating characteristics curves were generated for each cut-off point of the EVE index, and the cut-off points

Table 1 Baseline characteristics of patients receiving peginterferon α-2b plus ribavirin for genotype lb with a high viral load

	Sustained virologic responders ($n = 63$)	Relapsers ($n = 22$)	P
Age (years)	55 ± 12	61 ± 6	0.027
Gender, n (%)			
Female	22 (35)	10 (45)	0.38
Male	41 (65)	12 (55)	
Virus loa (Log IU/mL)	5.8 ± 0.6	6.0 ± 0.3	0.23
Treated, n (%)	23 (37)	8 (36)	0.99
Body weight (kg)	60.6 ± 14.5	60.2 ± 10.4	0.84
Body mass index (kg/m^2)	23.0 ± 3.1	23.6 ± 3.3	0.49
Hemoglobin (g/dL)	14.2 ± 1.3	14.1 ± 1.2	0.57
Platelets (×10^4/μL)	16.8 ± 4.5	14.9 ± 3.2	0.07
ALT (IU/mL)	84 ± 82	71 ± 84	0.12
Creatinine level (mg/dL)	0.66 ± 0.20	0.60 ± 0.27	0.52
Fibrosis stage, n (%)			
F0F1	23	1	0.0045
F2F3	32	17	
Unknown	8	4	

Results are presented as mean ± standard deviation. ALT, alaizine aininotransferase.

were set so that SVR had a probability of 95%. The area under each receiver operating characteristics curve was calculated to assess the extent of discrimination provided by these parameters.

This study was carried out in accordance with the Helsinki Declaration, and was approved by the Human Ethics Review Committee of Hiroshima University and the ethics committees of each center. All patients provided written informed consent.

RESULTS

STANDARD THERAPY WITH Peg-IFNα2b plus RBV was performed in 106 chronic HCV patients with genotype 1b and a high viral load between January and December 2005. After excluding the subjects who were administered less than 80% of the scheduled combination dose of Peg-IFNα2b and RBV, there were 96 patients (90.6%) who completed the 48-week treatment period. Among them, 85 patients (80.2%) and 63 patients (59.4%) showed ETR and SVR, respectively, while 22 patients (20.8%) suffered from relapse and became positive for HCV RNA again within 24 weeks after the end of treatment.

Baseline characteristics of the SVR group and relapse group

To predict SVR and relapse, comparison of the two groups was performed with respect to various baseline characteristics, including age, sex, viral load, prior IFN therapy, body weight, body mass index, hemoglobin, platelet count, alanine aminotransferase, creatinine, and liver histology (F0 or F1). Patients were younger in the SVR group than in the relapse group ($P = 0.027$), and the incidence of F0 or F1 liver fibrosis was higher in the SVR group ($P = 0.0045$) (Table 1).

EVE index in the SVR and relapse groups

The mean R^2 value (coefficient of determination) was 0.97, when changes in the viral load of each patient until HCV became negative were described by an approximate exponential curve to calculate the EVE index.

Figure 2 displays the EVE index in each group using dots and box-and-whisker plots. The EVE index was significantly lower in the SVR group than in the relapse group (0.022 ± 0.048 vs. 0.30 ± 0.29, $P < 0.0001$).

SVR was noted 0% ($n = 0$) of patients when the EVE index was 1 to less than 10 ($n = 1$), 16.7% ($n = 3$) when it was 10^{-1} to less than 1 ($n = 18$), 73.7% ($n = 14$) when it was 10^{-2} to less than 10^{-1} ($n = 19$), 96.4% ($n = 27$) when it was 10^{-3} to less than 10^{-2} ($n = 28$), and 100% ($n = 19$) when it was 10^{-3} or less ($n = 19$). Receiver operating characteristics (ROC) analysis showed that the AUC value discriminating SVR from relapse based on the EVE index was 0.94. When the cut-off point of the EVE index was set at

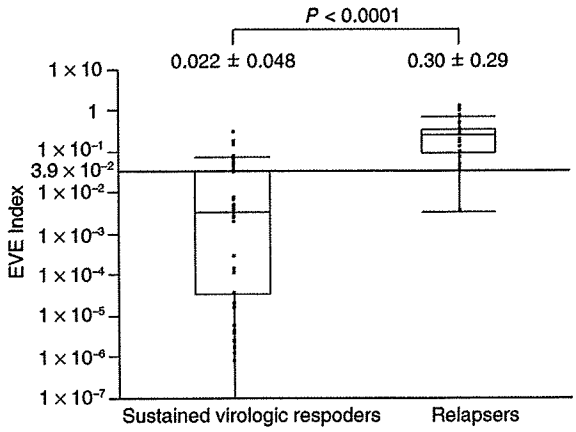

Figure 2 Box plot of the end of treatment (EVE) index in the sustained virologic response and relapse groups.

3.9×10^{-2} or less (Fig. 2), the positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, and accuracy for SVR were 96.2%, 60.6%, 79.3%, 90.9%, and 82.3%, respectively. On the other hand, SVR was only noted in 39.4% of patients in whom the predicted load was above the cut-off point. When SVR was estimated from viral dynamics at the time when serum HCV RNA became negative after 12 weeks of treatment, the PPV, NPV, sensitivity, specificity, and accuracy for SVR were 92.2%, 81.0%, 93.7%, 77.3%, and 79.0%, respectively. When SVR was estimated from viral dynamics at the time of a decrease of serum HCV RNA by at least 2 log units (4 weeks/0 weeks), the PPV, NPV, sensitivity, specificity, and accuracy were 82.5%, 50.0%, 82.5%, 50.0%, and 64.9%, respectively (Table 2).

Relationship between the cut-off point and SVR stratified by the timing of HCV RNA negativity in each group

All of the patients showing RVR ($n = 14$) also had SVR, and the EVE index was below the cut-off point in all 14 patients. Among the patients with cEVR ($n = 50$), SVR was noted in 90.0%. In this group, SVR was achieved in 75.0% of patients with an EVE index above the cut-off point, while it was achieved in 94.7% of patients with an index below the cut-off point ($P = 0.047$). Among the patients showing LVR ($n = 21$), SVR was achieved in 19.0%, and no patient had an EVE index that was below the cut-off point (Fig. 3).

Relationship between the cut-off point and SVR stratified by the time when HCV RNA showed a 2-log or more decrease in each group

When the HCV RNA load showed a 2-log or more decrease after 1 or 2 weeks of treatment (1 week: $n = 5$, 2 weeks: $n = 10$), SVR was noted in all 15 patients and the EVE index was below the cut-off point in all of them. When the HCV RNA load showed a 2-log or more decrease after 4 weeks of treatment, SVR was achieved in 77.1%. It was noted in 43.8% of patients in whom the EVE index was above the cut-off point, while it was seen in 93.8% of those in whom the index was below the cut-off point ($P < 0.0001$). When the HCV RNA load showed a 2-log or more decrease after 8 weeks of treatment, SVR was noted in 52.6%. It was seen in 25.0% of patients in whom the EVE index was above the cut-off point, while it was achieved in all of the patients in whom the index was below the cut-off point ($P = 0.002$). When the HCV RNA load showed a 2-log or more decrease after 12 weeks of treatment, SVR was noted in 33.3%, and there was no patient in whom the EVE index was below the cut-off point (Fig. 4).

DISCUSSION

THE PRESENT STUDY revealed that when combination therapy with Peg-IFNα2b plus RBV is given to HCV patients with genotype 1b and a high viral load, it

Table 2 Predicting sustained virologic response (SVR) using end of treatment (EVE) index

Criteria for SVR prediction	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	accuracy (%)
EVE index ≤ 3.9×10^{-2}	96.2	60.6	79.3	90.9	82.3
Negative for serum HCV RNA after 12 weeks of treatment	92.2	81.0	93.7	77.3	79.0
The ratio of decrease in serum HCV RNA (4 week/0 week) >2 logs	82.5	50.0	82.5	50.0	64.9

HCV, hepatitis C virus; NPV, negative predictive value (% not meeting the criteria for SVR prediction that were relapse); PPV, positive predictive value (% meeting the criteria for SVR prediction that were SVR).

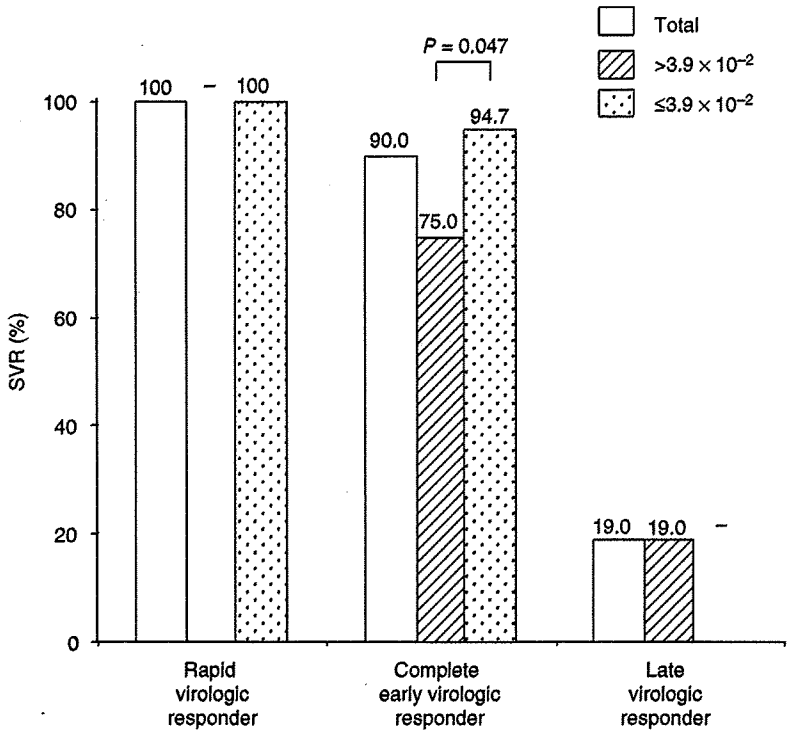

Figure 3 Sustained virologic response (SVR) rate stratified by the time when serum hepatitis C virus RNA became negate whom the end of treatment index was lower or higher than the cut-off point ($\leq 3.9 \times 10^{-2}$).

is possible to predict SVR or relapse by using the EVE index.

In recent years, several reports have been published concerning prediction of the efficacy of combination therapy with Peg-IFN plus RBV. In some studies, treatment was discontinued if the early virologic response suggested that interferon therapy would not achieve SVR, while an attempt was made to select an appropriate

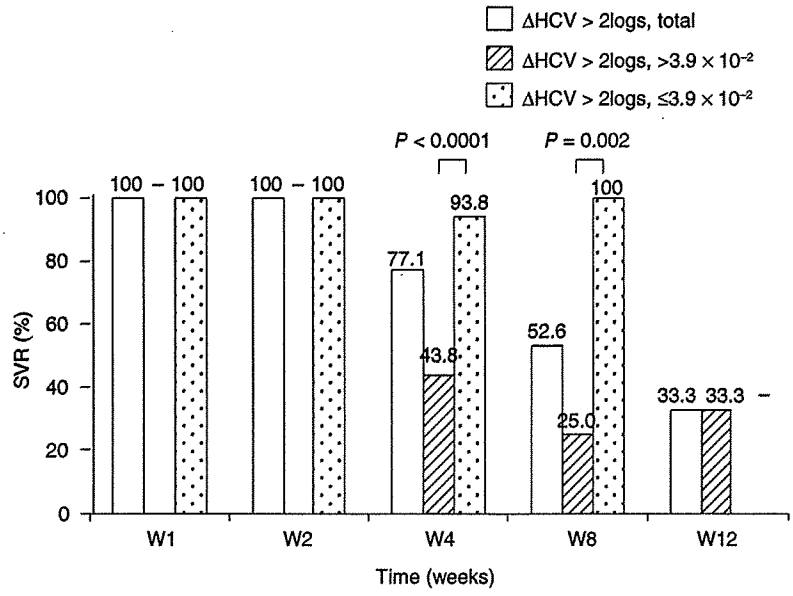

Figure 4 Relationship between the cut-off point ($\leq 3.9 \times 10^{-2}$) and the sustained virologic response rate when serum hepatitis C virus (HCV) RNA showed a 2-log or more decrease in each group.

treatment period for prevention of relapse and to increase the SVR rate if interferon was considered likely to achieve SVR.[4,6–12] Attempts have also been made to shorten the treatment period to 24 weeks in RVR patients showing a very good early virologic response.[4,12–14]

However, the above studies did not consider differences in the baseline viral load between individual patients because the efficacy of interferon therapy was estimated from viral dynamics at the time when serum HCV RNA became negative or the time when serum HCV RNA showed a 2-log or more decrease. Moreover, comparison was only done between two points (i.e. before treatment and at each time of testing). On the other hand, the accuracy of estimation was higher in the present study, because the response was predicted from the viral load profile obtained by frequent measurement from before treatment until the time when HCV RNA became negative. Frequent measurement was performed because even among patients who show a negative result for HCV-RNA or a 2-log or more decrease after the same period, the slope of the decrease of the viral load until it becomes negative may vary due to differences of the load before or during treatment, and this might lead to differences of the HCV RNA load at the end of treatment. Time and the viral load were plotted on the abscissa and ordinate, respectively, and the decrease of viral load until HCV RNA became negative was approximated by an exponential curve, which was then extended to estimate the viral load after serum HCV RNA became negative and also the slope of its decrease. According to our results, the SVR rate showed a significant difference in relation to whether or not the EVE index was below the cut-off point among patients showing EVR and also among patients in whom the HCV RNA load first showed a 2-log or more decrease after 4 or 8 weeks of treatment (Figs 3,4). These results suggested that the accuracy of predicting the response from the EVE index is higher than the conventional method based on the time when serum HCV RNA becomes negative or the time when HCV RNA shows a 2-log or more decrease. Another advantage of our method is that the SVR is not only predictable at a limited number of times (such as 24 and 48 weeks) after HCV RNA becomes negative, but also at the end of any specified treatment period, because the viral load can be calculated for any duration of treatment after serum HCV RNA becomes negative.

The main limitation of our method is difficulty in predicting efficacy because viral kinetics would deviate from the decay curve for the EVE index if a marked change is made to the dose of Peg-IFN and/or RBV after the start of treatment. This is also a problem when predicting the therapeutic efficacy at the time of viral negativity and the period when the viral load is reduced to 2 log or less. Prediction of efficacy would become further difficult depending on the changes in the amount or timing of drug administration after determining the predicted dose. None of the subjects had marked changes of the dosage in this study because subjects with <80% adherence to the Peg-IFN and RBV regimen were excluded. The EVE index is a useful method for predicting efficacy when administration of the standard dose is continued.

The viral dynamics during combination therapy with Peg-IFN plus RBV in chronic hepatitis C patients can be divided into 3 phases, which are the first phase (1–2 days) with a rapid decline of the viral load, followed by the "shoulder phase" (4–28 days) in which viral load declines slowly or remains constant, and the third phase of a further decrease in the load. In recent years, each of these 3 phases of viral dynamics has been reported to be useful for predicting the response to IFN therapy in patients with hepatitis C.[17–22] In the model of Herrmann *et al.*, the slope of the "shoulder phase" in patients with triphasic viral decay represents the pretreatment death rate of infected cells and the slope during the third phase represents the treatment-enhanced death rate of infected cells due to the immunomodulatory effect of RBV.[17] In contrast, Dahari *et al.* stated that the shoulder phase does not represent the intrinsic death rate of infected cells, and they considered the third-phase slope to be close to the intrinsic death rate of these cells.[18] The curves generated in the present study represent viral dynamics from the first to third phases as a continuous curve based on exponential approximation (indefinite phase) rather than as three lines (Fig. 1b). For easier clinical use, index approximation was applied. As a result, the mean R^2 value (coefficient of determination) calculated for our approximate exponential curve was 0.97, which indicated a strong correlation.

In conclusion, predicting the response from the EVE index is more useful than conventional estimation from the viral load profile at the time when serum HCV RNA becomes negative or when serum HCV RNA shows a 2-log or more decrease. Using our method, it is possible to calculate the EVE index any time during treatment or for any treatment period. This may help to improve the motivation of patients who have been treated for a long period while suffering from adverse reactions.

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Randomized Trial of High-Dose Interferon-α-2b Combined With Ribavirin in Patients With Chronic Hepatitis C: Correlation Between Amino Acid Substitutions in the Core/NS5A Region and Virological Response to Interferon Therapy

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The aim of this study was to compare the efficacy of high-dose interferon (IFN)-α-2b with standard dose of IFN-α-2b in combination with ribavirin (RBV) for patients with chronic hepatitis C virus (HCV) infection, and to investigate the predictive factors associated with virological response. Two hundred Japanese patients with high HCV viral load (>100 KIU/ml) were randomized to 6 or 10 mega units (MU) of 24-week IFN-α-2b with RBV. Predictive factors were investigated; including pretreatment amino acid (aa) sequences of the core region and the IFN-sensitive determining region (ISDR). The sustained virological response rate was not different in the two groups (24% vs. 30%) but the incidence of depression was significantly higher in the 10 MU group than 6 MU group (7% vs. 0%, $P=0.02$). Younger age (<60) and HCV genotype (2a/b) were significant predictors of sustained virological response. In patients infected with genotype 1b, substitutions of core aa 70 and/or 91 were predictive for non-virological response ($P<0.001$), and substitutions in the ISDR was observed frequently in virological responders. Early viral kinetics study showed that serum HCV core antigen decreased more slowly in both patients with aa 70 and/or 91 substitutions in the core and with absence of substitutions in the ISDR. In conclusion, the use of a higher dose of IFN-α-2b in combination with RBV did not improve virological response but resulted in higher incidence of depression. Amino acid substitutions in the core and ISDR are predictive of virological response to the therapy in patients with genotype 1b and high viral load. ***J. Med. Virol. 81:640–649, 2009.***

KEY WORDS: HCV; interferon; ribavirin; core region; ISDR

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is the leading cause of cirrhosis, liver failure, and hepatocellular carcinoma [Kiyosawa et al., 1990; Niederau et al., 1998]. Interferon (IFN) is an essential component of therapy for patients with chronic HCV infection. The most effective therapy available at present is the combination therapy of pegylated (PEG)-IFN and ribavirin (RBV) [Manns et al., 2001; Fried et al., 2002; Hoofnagle et al., 2003]. Among HCV genotypes, genotype 1b is the most resistant genotype to IFN therapy [Fried et al., 2002]. The limitation of use of the combination therapy for HCV infection with genotype 1b is due to the low response rate during therapy and high relapse rate after the therapy [McHutchison et al., 1998]. Several studies have evaluated the potential benefits of a larger dose of IFN with varying results [Lindsay et al., 1996; Fried et al., 2000; Ferenci et al., 2001; Hadziyannis et al., 2001; Di Marco et al., 2002; Brouwer et al., 2004]. Although treatment has been

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

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

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

PATIENTS AND METHODS

Patient Selection

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

Study Design

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

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

Assessment of Efficacy

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

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

Nucleotide Sequencing of the Core and NS5A Gene

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

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

Quantitation of HCV Core Antigen

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

Statistical Analysis

The baseline characteristics of the patients in the two groups were compared and the differences were assessed by Chi-square test with Yate's correction and Mann–Whitney *U*-test. To assess the sustained virological response rates, an intention-to-treat (ITT) analysis and a per-protocol (PP) analysis were conducted. The response rates and substitutions in the core region and the ISDR were compared by Fisher's exact test. All *P* values reported are two-sided and those less than 0.05 were considered significant. To determine the predictors of sustained virological and non-virological responses, univariate and multivariate logistic regression analyses were carried out. Potential predictive factors included the following variables: age, sex, alcohol consumption, past history of IFN monotherapy, body mass index, ALT, hemoglobin, platelets, HCV RNA level, genotype, liver histology, total RBV dose (adjusted for body weight [mg/kg]) and total dose of IFN-α-2b. The odds ratio and 95% confidence intervals (95% CI) were also calculated. Variables with statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

Patient Demographics

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

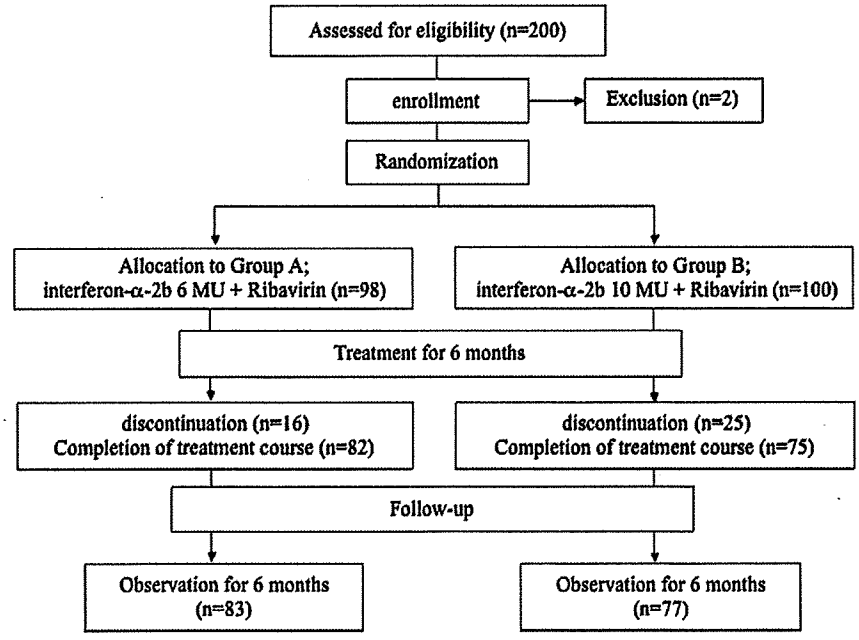

Fig. 1. Flow chart of number of patients throughout the trial. A total of 200 patients were included in this study. One hundred ninety-eight patients met the eligibility criteria and they underwent randomization, 98 patients in Group A and 100 patients in Group B.

TABLE I. Baseline Characteristics of the Patients

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

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

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

Overall Sustained Virological Response

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

Dose Reduction or Discontinuation and Adverse Events

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

Predictive Factors Associated With Sustained Virological Response

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

TABLE II. Rates of Sustained Virological Response According to Adherence

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

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

TABLE III. Dose Reduction or Discontinuation and Adverse Events

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

IFN, interferon; RBV, ribavirin.

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

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

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

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

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

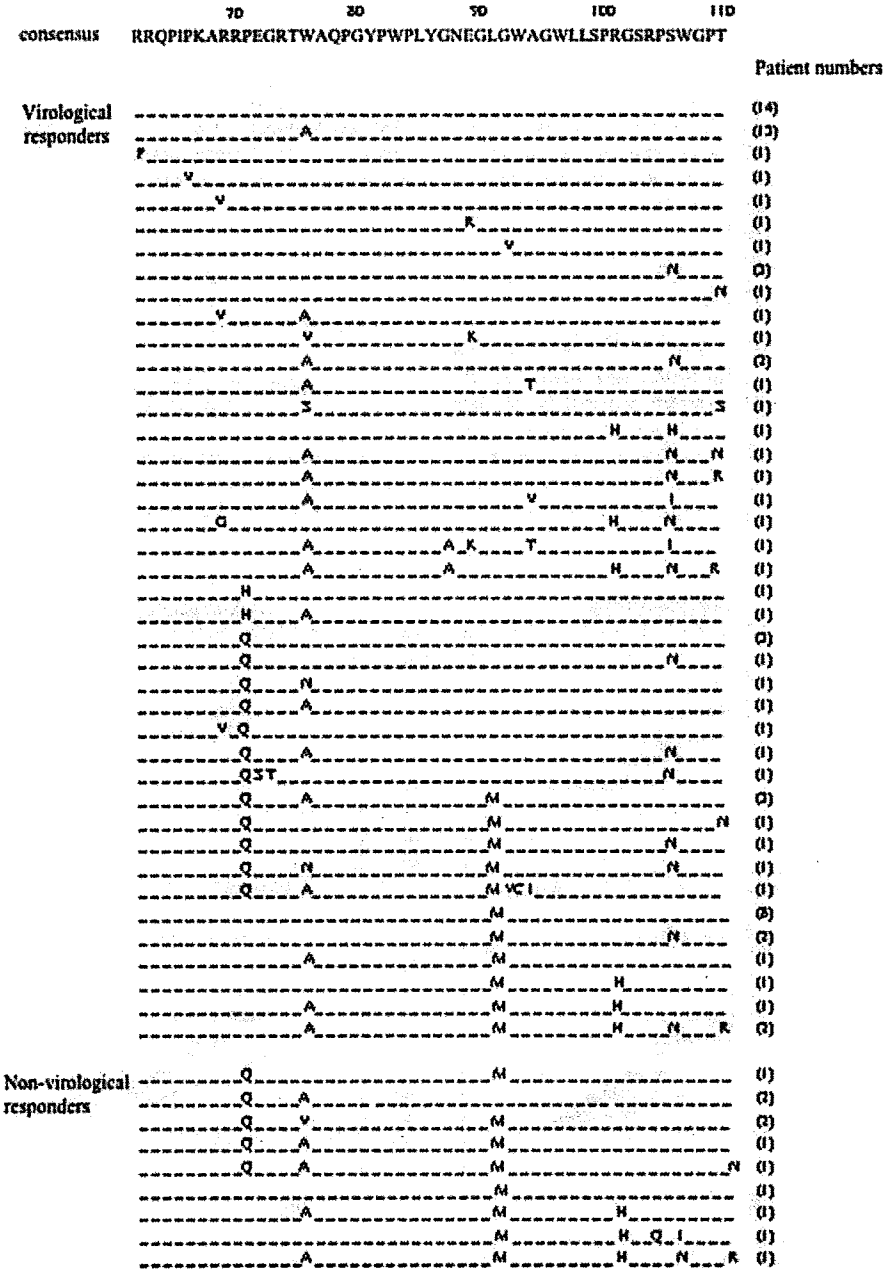

Fig. 2. Sequences of amino acids 61–110 in the core region at commencement of combination therapy in 93 patients infected with hepatitis C virus genotype 1b. Dashes indicate amino acids identical to the consensus sequence of genotype 1b, and substituted amino acids are shown by standard single-letter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

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

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

aa, amino acid.

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

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

Analysis of Nucleotide Sequence of the NS5A Gene

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

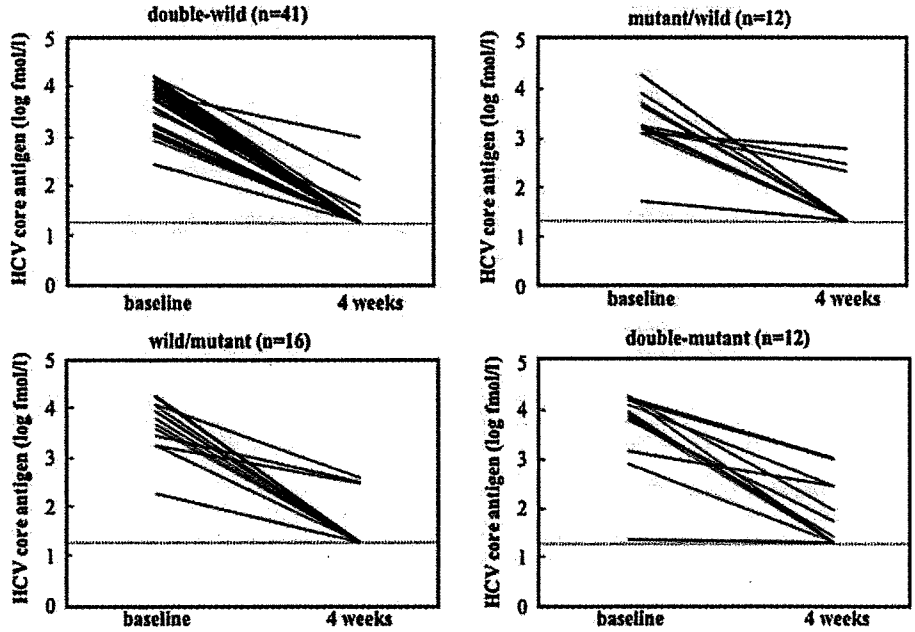

Fig. 3. Reduction of amount of HCV core antigen based on the presence of substitutions at amino acid 70 or 91. Eighty-one patients infected with hepatitis C virus were treated with combination therapy. Serum HCV core antigen was measured before treatment (baseline) and at week 4. The response was divided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

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

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

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

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

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

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

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

DISCUSSION

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

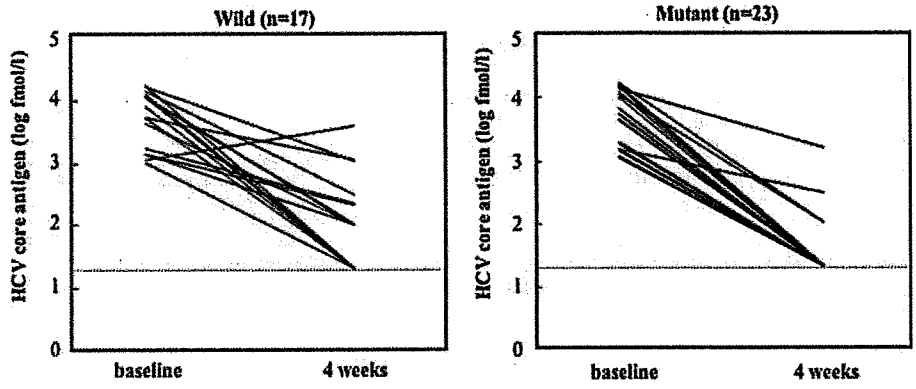

Fig. 4. Reduction of amount of HCV core antigen based on the presence of substitutions in the ISDR. Sixty-five patients infected with hepatitis C virus were treated with combination therapy. Serum HCV core antigen was measured before treatment (baseline) and at week 4. Patients were divided into two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

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

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

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

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

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

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

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

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

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

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A Polymorphism in *MAPKAPK3* Affects Response to Interferon Therapy for Chronic Hepatitis C

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Background & Aims: **This study aimed to identify host single nucleotide polymorphisms (SNPs) that are associated with the efficacy of interferon (IFN) therapy in patients with chronic hepatitis C.** ***Methods:*** **We examined whether 116 tagging-SNPs from 13 genes that are involved in type I IFN signaling associate with the outcome of IFN therapy in Japanese case-control groups; the study included 468 sustained responders and 587 nonresponders.** ***Results:*** **We identified 2 SNPs (rs3792323 [A/T] and rs616589 [G/A]), located in intron 2 of mitogen-activated protein kinase–activated protein kinase 3 (MAPKAPK3) that were associated with the outcome of IFN therapy in patients infected with hepatitis C virus (HCV) genotype 1b ($P = 4.6 \times 10^{-5}$ and 4.8×10^{-5}, respectively). The 2 SNPs were in strong linkage disequilibrium and multivariate logistic regression analysis showed that rs3792323 is an independent factor associated with the IFN efficacy (genotype 1b; $P = .0011$). *MAPKAPK3* is a kinase involved in the mitogen and stress responses, but the biological significance of MAPKAPK3 in IFN responses is poorly understood. By using an allele-specific transcript quantification assay in liver biopsy, we showed that allele-specific expression of *MAPKAPK3* messenger RNA, corresponding to the risk allele for nonresponse, was significantly higher than that of the other allele. Luciferase reporter assay data indicated that overexpression of MAPKAPK3 inhibits IFN-alfa–induced gene transcription via IFN-stimulated response element and IFN-γ–activated site.** ***Conclusions:*** **The SNP rs3792323 in *MAPKAPK3* associates with the outcome of IFN therapy in patients with HCV genotype 1b. Our functional analyses indicate that MAPKAPK3 inhibits IFN-alfa–induced antiviral activity.**

Type I interferon (IFN), including IFN-alfa and IFN-β, has been used widely as an antiviral agent for chronic hepatitis C. However, even after the most effective combination therapy of pegylated-IFN-alfa plus ribavirin, more than 50% of patients infected with hepatitis C virus (HCV) genotype 1b and approximately 20% of patients with HCV genotype non-1b fail to eradicate the virus.[1–3]

The mechanisms of modulating the responsiveness to IFN therapy have been studied extensively. Both viral and host factors have been implicated in the resistance to IFN therapy. Viral factors, such as HCV genotype, serum HCV-RNA level, and the interferon sensitivity determining region, have been reported to be associated with the outcome of IFN therapy.[3–5] On the other hand, host factors including age, sex, race, liver fibrosis, and obesity have been shown to associate with the outcome of IFN therapy.[6,7] Furthermore, it has been reported that genetic polymorphisms of cytokines, chemokines, and IFN-stimulated genes are associated with the difference in response to IFN therapy.[7–12]

Recently, genetic polymorphism of type I IFN receptor-1 (IFNAR1) promoter region was reported to be associated with the outcome of IFN therapy in patients with HCV infection.[13] Although the mechanisms of this polymorphism for the different responsiveness to IFN therapy still are unclear, polymorphism of *IFNAR1* promoter region may influence the efficacy of IFN therapy, possibly through modulation of IFNAR1 expression level. Because type I IFN elicits antiviral activity by activation of signaling molecules downstream of type I IFN receptors, genetic polymorphisms in type I IFN signaling molecules

Abbreviations used in this paper: GAS, interferon-γ–activated site; IFN, interferon; IFNAR1, type I interferon receptor-1; ISRE, interferon-stimulated response element; JAK, Janus-activated kinase; MAP, mitogen-activated protein; MAPKAPK, mitogen-activated protein kinase–activated protein kinase; MAPKK, mitogen-activated protein kinase kinases; NR, nonresponders; SNPs, single nucleotide polymorphisms; SR, sustained responders; STAT, signal transducer and activator of transcription.

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