Outcome and Early Viral Dynamics with Viral Mutation in PEG-IFN/RBV Therapy for Chronic Hepatitis in Patients with High Viral Loads of Serum HCV RNA Genotype 1b

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Key Words

Chronic hepatitis • Early viral dynamics • IFN/RBV resistance-determining region • HCV RNA genotype 1b • High viral load • PEG-IFN/RBV combination therapy • Virological response, prediction

Abstract

We investigated whether sustained virological response (SVR) and non-SVR by chronic hepatitis C patients to pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy are distinguishable by viral factors such as the IFN/RBV resistance-determining region (IRRDR) and by on-treatment factors through new indices such as the rebound index (RI). The first RI (RI-1st; the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd; the viral load at week 2 divided by the viral load at 24 h) were calculated. The subject patients were divided into 3 groups based on RI-1st and RI-2nd: an RI-A group (RI-1st ≤ 1.0), an RI-B group (RI-1st > 1.0 and RI-2nd < 0.7) and an RI-C group (RI-1st > 1.0 and RI-2nd ≥ 0.7). The SVR rate was 71.4% (10/14) in the RI-A group,

46.2% (6/13) in the RI-B group and 20.0% (3/15) in the RI-C group ($p = 0.005$ between the RI-A group and the RI-C group). In IRRDR ≥ 6 and IRRDR ≤ 5 the SVR rate was 81.3% (13/16) and 23.1% (6/26) ($p = 0.0002$), respectively. By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group (≥ 6 mutations in the IRRDR) and 7.7% (1/13) in the RI-C group (≤ 5 IRRDR mutations) ($p = 0.0003$).

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Introduction

Recently, global consensus has obtained that a combination of IFN or pegylated IFN plus ribavirin (PEG-IFN/RBV) is the treatment of choice for chronic hepatitis C (CHC). Notwithstanding this treatment regimen, sustained virological response (SVR) rates of those infected with the most resistant genotypes [hepatitis C virus (HCV)-1a and -1b] still hover at $\sim 50\%$ [1, 2]. It is therefore worthwhile to identify the predictive factors that allow the selection of patients who would achieve eradication

of HCV RNA either before or during therapy, especially since IFN/RBV combination therapy is costly and has several side effects [3].

Predictors of the effectiveness of IFN-based therapy can be classified into pretreatment and on-treatment factors. Pretreatment factors comprise: (1) host factors such as age, gender, obesity, alcohol consumption, hepatic iron overload, fibrosis, immune responses and co-infection with other viruses, and (2) viral factors that mainly include viral genotypes and loads, particular amino acid sequence variations in the NS5A region [4, 5] and in the core protein region of HCV [6] within a given genotype. Moreover, the mean number of mutations in variable region 3 (V3) plus its upstream flanking region of NS5A [amino acid 2334–2379, referred to as IFN/RBV resistance-determining region (IRRDR)] is significantly higher in HCV isolates obtained from patients who later achieve SVR by PEG-IFN/RBV than in those from non-SVR patients. On-treatment factors are mainly related to viral kinetics within the first few weeks of treatment [7].

In the current study, with the aim of investigating whether SVR and non-SVR can be distinguished by viral factors such as IRRDR and by on-treatment factors through new indices such as the rebound index (RI), we calculated the first RI (RI-1st; the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd; the viral load at week 2 divided by the viral load at 24 h), as proposed by Nomura et al. [8].

Patients and Methods

The 42 patients included in this study, who all demonstrated high viral loads (>100 KIU/ml) of serum HCV RNA of genotype 1b, had been diagnosed with CHC on the basis of abnormal serum alanine aminotransferase persisting for at least 6 months, and of positive HCV RNA assessed by RT-PCR. None of the patients was positive for hepatitis B surface antigen or other liver diseases (autoimmune hepatitis, alcoholic liver disease). All the patients received a regimen of PEG-IFN α -2b (peginterferon alpha-2b; Peg-Intron; Schering-Plough, Kenilworth, N.J., USA) (1.5 μ g/kg/week, subcutaneously) in combination with RBV (ribavirin; Rebetol; Schering-Plough) 600–1,000 mg/day for 48 weeks. RBV was administered at a dose of 600 mg/day (3 capsules) to patients weighing <60 kg, 800 mg/day (4 capsules) to those weighing <80 kg and 1,000 mg/day (5 capsules) to those weighing ≥ 80 kg.

The efficacy of the combination therapy was evaluated by HCV RNA negativity determined by qualitative RT-PCR analysis at the end of therapy (end of therapy response) and 6 months after the completion of therapy (SVR). The amount of HCV RNA was also measured quantitatively by RT-PCR (Amplicor HCV monitor v. 2.0; Roche) before therapy. The lower detection limit of the assay was 5 KIU/ml. Samples collected during and after therapy

were also determined by qualitative RT-PCR (Amplicor; Roche), which has a higher sensitivity than quantitative analysis, and the results were labeled as positive or negative. The lower limit of the assay was 50 IU/ml.

SVR was defined as undetectable serum HCV RNA at 24 weeks after the cessation of treatment, and non-SVR as detectable HCV RNA at 24 weeks after the discontinuation of treatment. Informed consent was obtained from all patients enrolled in the study after thoroughly explaining the aims, risks and benefits of the therapy.

The amount of HCV core antigen was assessed by the IRM assay (Ortho Clinical Diagnostics, Tokyo, Japan), which provides a good correlation between the amount of HCV core antigen and the amount of HCV RNA, as shown in our previous study [9]. The HCV core antigen was measured on days 0, 1 (24 h), 7 (1 week) and 14 (2 weeks) according to the detection limit of 20 fmol/l established by the manufacturer.

RI-1st was defined as the coefficient derived by dividing the viral load of HCV core antigen at week 1 by that at 24 h, and RI-2nd was defined as the coefficient derived by dividing the viral load at week 2 by that at 24 h [8].

The patients were divided into 3 groups based on RI-1st and RI-2nd: group A (RI-1st ≤ 1.0), group B (RI-1st >1.0 and RI-2nd <0.7) and group C (RI-1st >1.0 and RI-2nd ≥ 0.7).

NS5A sequence analysis (IRRDR) was performed as described [4]. Briefly, the sequences of the amplified fragments were determined by direct sequencing without subcloning with the use of a Big Dye Deoxy Terminator cycle sequencing kit and an ABI 337 DNA sequencer (Applied Biosystems, Japan). The aa sequences were deduced and aligned with Genetyx Win software v. 7.0 (Genetyx Corp., Tokyo, Japan). Numbering of aa throughout the manuscript is according to the polyprotein of HCV genotype 1b prototype HCV-J.

Statistical Analysis

Differences between the groups were assessed by the χ^2 test, Fisher's exact test or Student's *t* test, the Mann-Whitney test and the Kruskal-Wallis test. $p < 0.05$ was considered statistically significant.

Results

Of the 42 patients treated with combination therapy, 19 (45.2%) achieved SVR and 23 (54.8%) were still HCV RNA positive (non-SVR) 6 months after therapy. No significant differences were observed in patient characteristics between SVR and non-SVR, except in platelet counts and the degree of fibrosis (table 1), or among the RI-A, -B and -C groups (table 2).

The SVR rate was 71.4% (10/14), 46.2% (6/13) and 20.0% (3/15) in the RI-A, -B and -C groups, respectively, with a significant difference between the RI-A and -C groups ($p = 0.005$), but not significant between the RI-A and -B groups and the RI-B and -C groups (fig. 1). In the 14 patients of the RI-A group, HCV RNA turned negative

Table 1. Host-dependent, virus-related profile by response (SVR and non-SVR)

	SVR	Non-SVR	p value
Gender (M/F), n	11/8	13/10	NS
Age, years	56.7 ± 8.8	59.3 ± 10.5	NS
HCV RNA level, KIU/ml	1,685 ± 1,477	1,660 ± 1,363	NS
HCV core antigen, fmol/l	7,044 ± 6,763	9,343 ± 12,563	NS
Body weight, kg	59.9 ± 11.5	59.8 ± 13.6	NS
Treatment history (retreatment/naïve)	6/13	13/10	NS
Platelet count (× 10 ⁴ /mm ³)	18.7 ± 4.4	14.8 ± 5.4	0.02
F0, 1/F2, 3	12/2	5/10	0.004

Table 2. Host-dependent, virus-related profile by response (RI-A, -B and -C groups)

	RI-A	RI-B	RI-C	p value
Gender (M/F), n	7/7	9/4	8/7	NS
Age, years	60.0 ± 5.9	58.5 ± 9.4	56.1 ± 12.8	NS
HCV RNA level, KIU/ml	1,401 ± 1,014	2,053 ± 1,286	1,593 ± 1,772	NS
HCV core antigen, fmol/l	6,084 ± 5,106	7,674 ± 5,038	11,000 ± 15,837	NS
Body weight, kg	62.1 ± 16.6	59.5 ± 10.4	58.2 ± 10.1	NS
Treatment history (retreatment/naïve)	3/11	7/6	9/6	NS
Platelet count (× 10 ⁴ /mm ³)	15.3 ± 3.5	18.3 ± 5.9	16.3 ± 6.0	NS
F0, 1/F2, 3	7/3	5/4	5/5	NS

Table 3. SVR rate between IRRDR ≤5 and IRRDR ≥6 in RI-A, -B and -C groups

	RI-A		RI-B		RI-C	
	IRRDR ≤5	IRRDR ≥6	IRRDR ≤5	IRRDR ≥6	IRRDR ≤5	IRRDR ≥6
SVR	3	7	2	4	1	2
Non-SVR	3	1	5	2	12	0
SVR rate, %	50.0	87.5	28.6	66.7	7.7	100
p value	NS		NS		0.0024	
	0.0003					

by week 4 in 3 patients, week 8 in 5 patients, week 12 in 5 patients and was positive in 1 patient throughout the treatment. In the 13 patients of the RI-B group, HCV RNA was negative by week 4 in 1 patient, week 8 in 2 patients, week 12 in 4 patients, at and after week 16 in 5 patients and remained positive throughout the treatment in 1 patient. In the 15 patients of the RI-C group, HCV RNA was negative by week 12 in 1 patient, on and after week 16 in 6 patients and remained positive throughout the treatment in 8 patients (fig. 2).

The SVR rate was 81.3% (13/16) in the group with ≥6 mutations in IRRDR, and 23.1% (6/26) in those with ≤5 (fig. 3), with a significant difference between the 2 groups ($p = 0.0002$).

By combining RI and IRRDR, the SVR rate was 87.5% (7/8) in the RI-A group (IRRDR ≥6) and 7.7% (1/13) in the RI-C group (IRRDR ≤5) (table 3), with a significant difference between the 2 groups ($p = 0.0003$).

Fig. 1. SVR rate in RI-A, -B and -C groups. The overall SVR rate was 71.4, 46.2 and 20.0%, respectively. Significant difference in SVR rate is indicated.

Fig. 3. SVR rate and IRRDR number. The SVR rate was 23.1% in IRRDR ≤5 and 81.3% in IRRDR ≥6, which was significantly different.

Fig. 2. Relation between response time and virus dynamics. In the 14 patients of the RI-A group, HCV RNA turned negative by week 4 in 3 patients, week 8 in 5 patients, week 12 in 5 patients and remained positive throughout the treatment in 1 patient. In the 13 patients of the RI-B group, HCV RNA was negative by week 4 in 1 patient, week 8 in 2 patients, week 12 in 4 patients, at and after week 16 in 5 patients and remained positive throughout the treatment in 1 patient. In the 15 patients of the RI-C group, HCV RNA was negative by week 12 in 1 patient, at and after week 16 in 6 patients and remained positive throughout the treatment in 8 patients.

Discussion

The importance of early virological response (EVR; signifying HCV RNA negative at 12 weeks) has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those not reaching EVR do not respond to further therapy. Discontinuation of treatment in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is worth investigating [10].

Treatment with IFN induces a decline in HCV RNA levels that can be mathematically measured in 2 phases. The decline in the first phase, usually measured at 24 or 48 h, probably reflects direct inhibition of intracellular production and release of HCV [11], with IFN efficacy ranging from about 70% (approx. 0.7 log units) for standard IFN (given 3 times a week) to more than 90% (1 log unit) for high daily doses of standard IFN or PEG-IFN (given once a week) [12, 13]. The decline in the second phase, beginning after 24–48 h, is slower and more variable than that in the first phase, and is thought to reflect continued inhibition of replication and the gradual elimination of virus-infected cells [11]. The decay in the first phase has little correlation with the IFN dose, but is more rapid with PEG-IFN than with standard IFN preparations [10].

Lowering HCV RNA during the first phase is essential for efficient elimination of HCV during the second phase. Decreases in HCV RNA titers within the first 24–48 h after the start of IFN would, therefore, be a dependable estimate of antiviral efficacy [12, 13].

Early viral kinetics, determined up to week 2, are believed to express the therapeutic effect of PEG-IFN. The concentration of PEG-IFN α -2b in serum peaks after 24 h, then declines gradually [14, 15]. The viral load is thus reduced by 24 h but increases in week 1 [16, 17]; with a large dose of PEG-IFN at each administration, it decreases markedly at 24 h but then increases in week 1 regardless of the dose. In the responder group, however, the viral load continues to decline each week thereafter [17].

In this study, we used new indices proposed by Nomura et al. [8]: RI-1st and RI-2nd calculated from early viral kinetics. RI-1st was defined as the coefficient derived by dividing the viral load of HCV core antigen at week 1 by that at 24 h, and the RI-2nd was defined as the coefficient derived by dividing the viral load at week 2 by that at 24 h. In the SVR group, a number of patients demonstrated no increase in the viral load at week 1. Patients with a high RI-2nd were regarded as poor responders or non-responders to PEG-IFN. The RI-2nd of those other than non-responders was below 0.7; therefore, 0.7 was adopted as the reference value for RI-2nd, and the patients were divided into 3 groups based on RI-1st and RI-2nd: the RI-A group (RI-1st ≤ 1.0), the RI-B group (RI-1st > 1.0 and RI-2nd < 0.7) and the RI-C group (RI-1st > 1.0 and RI-2nd ≥ 0.7). The SVR rate of the RI-A, RI-B and RI-C groups was 71.4% (10/14), 46.2% (6/13) and 20% (2/10), respectively ($p = 0.005$ between the RI-A group and the RI-C group). RIs are also associated with the early clearance of HCV RNA that is related to SVR.

In the RI-A group 21.4% (3/14), 35.7% (5/14) and 35.7% (5/14) became HCV RNA negative by weeks 4, 8 and 12, respectively. In the RI-B group 7.7% (1/13), 15.4% (2/13), 30.8% (4/13) and 38.5% (5/13) became HCV RNA negative by weeks 4, 8, 12, and at and after week 16, respectively. In the RI-C group 6.7% (1/15) and 40.0% (6/15) became HCV RNA negative by week 12, and at and after week 16, respectively. It is believed that the simplified RI-1st and RI-2nd are evidential indices for determining the therapeutic efficacy of PEG-IFN/RBV treatment.

We have previously reported that the high degree of sequence variation in IRRDR (IRRDR ≥ 6) significantly correlates with SVR, whereas the low degree of sequence variation in this region (IRRDR ≤ 5) correlates with non-SVR [4]. A significant correlation between the rapid reduction of HCV core antigen titers and the degree of se-

quence variation in IRRDR has been observed. This, in particular, suggests a possible influence of IRRDR ≥ 6 on HCV replication kinetics during IFN-based therapy, especially that the direct effect of IFN begins a few hours after the first dose.

In this study, the SVR rate was 81.2% (13/16) with IRRDR ≥ 6 and 23.1% (6/26) with IRRDR ≤ 5 ($p = 0.0002$), strongly suggesting that IRRDR ≥ 6 would be a useful marker for the prediction of SVR.

By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group (RI-1st ≤ 1.0) with IRRDR ≥ 6 , signifying that about 90% of these patients turned SVR and were, thus, believed to be very good responders. An SVR rate of 7.7% (1/13) was obtained in the RI-C group with IRRDR ≤ 5 ($p = 0.0003$).

In conclusion, we propose that IRRDR combined with RIs is the most sensitive predictive factor for SVR and non-SVR. With the aid of RIs and IRRDR, a more effective PEG-IFN/RBV treatment could be within reach. A more detailed investigation with a larger number of subjects is needed to confirm the current results in patients given PEG-IFN/RBV combination therapy.

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Disclosure Statement

No conflict of interest exists.

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