Early Viral Response Predicts the Efficacy of Antiviral Triple Therapy with Simeprevir, Peg-Interferon and Ribavirin in Patients Infected with Hepatitis C Virus Genotype 1

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Key Words
Chronic hepatitis C · Simeprevir · NS3/4 protease inhibitors · Interferon · Response-guided therapy

Abstract
Objectives: Triple therapy using peg-interferon, ribavirin and simeprevir (PEG-IFN/RBV/SMV) has reportedly resulted in high-sustained virological response (SVR) rates in patients with chronic hepatitis C (CHC), especially in naïve cases and relapers to prior PEG-IFN/RBV therapy. Here, we retrospectively analyzed the antiviral response associated with a triple regimen, in the context of early reduction of viral load during treatment. Methods: Forty-six CHC patients with HCV genotype 1b were treated with PEG-IFN/RBV/SMV triple therapy: 20 were naïve cases, 12 were relapers and 14 were non-responders to prior PEG-IFN/RBV therapy. We evaluated rapid virological response (RVR), complete early virological response (EVR), viral clearance at the end of the treatment (EOT) and at 12 weeks after the EOT (SVR12). In addition, we quantified the serum HCV-RNA on the 1st day and the 7th day after initiating treatment. Results: Multivariate analysis revealed that response to prior treatment was identified as an independent factor for achieving SVR12 after triple therapy (p = 0.0005). The achievement of serum HCV-RNA <2 log10 IU/ml on day 7, RVR, EVR and EOT were associated with SVR12 (p = 0.0050, p = 0.0002, p = 0.0009 and p = 0.0002, respectively). Conclusions: Rapid decline of HCV is a predictive factor for the achievement of SVR12, even in antiviral triple therapy with PEG-IFN/RBV/SMV. An extended treatment period should be applied for patients who show detectable serum HCV-RNA at week 4.

Introduction

Chronic hepatitis C (CHC) is one of the leading causes of liver cirrhosis worldwide and a major etiology for the emergence of hepatocellular carcinoma (HCC) [1]. Recent advancements in antiviral therapy for CHC allow us to have more treatment options, including triple therapy using peg-interferon (PEG-IFN), ribavirin (RBV) and NS3 protease inhibitors [2, 3]. Although IFN-free regimens are becoming more common, IFN-based regimens are still widely used for antiviral therapy of hepatitis C virus (HCV) because they can have a suppressive effect on the emergence of HCC and are associated with lower...
risk for developing virus mutations that are resistant to direct-acting antivirals (DAAs) [4, 5].

Simprevir (SMV) is a second-generation non-structural protein (NS) 3/4 protease inhibitor with a better safety profile than first-generation NS3/4 protease inhibitors; the latter, for example, are known to induce severe drug-induced eruptions [6]. Several reports have suggested that antiviral triple therapy with PEG-IFN, RBV and SMV (PEG-IFN/RNV/SMV) have shown powerful antiviral effects on HCV [2, 3, 7–9]. For example, 88.6% of patients in a study demonstrated sustained virological response 12 weeks after treatment end (SVR12) in treatment-naive cases with HCV genotype 1 infection and high viral load (HCV-RNA ≥5 log10 IU/ml) [10]. In addition, SVR12 was achieved in 95.9% of the patients who relapsed prior to receiving PEG-RFN and RBV combination therapy (PEG-IFN/RBV) and in 52.8% of prior non-responders (NRs) [11]. It has also been reported that PEG-IFN/RBV/SMV triple therapy may be effective after 24 weeks, compared to that after the standard 48-week regimen used in conventional PEG-IFN/RBV treatment [7]. Due to the potential efficacy of a PEG-IFN-based triple regimen, a treatment plan using triple therapy with SMV for 12 weeks, followed by additional administration of PEG-IFN/RBV for 12 weeks (SMV12/PR12), has been recommended for the treatment of naïve patients and prior relapsers. For prior NRs, an additional 36 weeks of administration of PEG-IFN/RBV after the initial 12 weeks of administration of 3 agents was also admitted (SMV12/PR36) [12].

Based on these findings, we conducted a retrospective analysis of the antiviral response of patients treated with a triple regimen of PEG-IFN/RBV/SMV, especially in the context of early reduction of viral load after treatment was initiated. For this purpose, we examined viral load intensively in the early phase of treatment.

Materials and Methods

Patients
Forty-six CHC patients infected with HCV genotype 1b were treated with PEG-IFN/RBV/SMV triple therapy between December 2013 and March 2015. All patients had a viral load of ≥5 log10 IU/ml before the treatment. The median age of the patients was 64.5 years, with a range of 33–81 years. Eighteen were men and 28 were women. Twenty-five patients were treated with PEG-IFN α-2a (Pegasys®, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), and 21 patients were administered PEG-IFN α-2b (PegIntron®, Merck Sharp & Dohme, Whitehouse Station, N.J., USA). Twenty-one showed IL-28B T/T genotype (rs8099917) and 12 were T/G, and data were missing for 13 patients. HCV with a mutation at the core amino acid 70 (core 70) was detected in 9 patients, while a wild mutation for core 70 was noted in 9 patients (data were missing for 19 patients). Of 46 patients, 20 were naïve cases, 12 were relapsers and 14 were NRs to prior treatment with PEG-IFN/RBV.

Assessment of Viral Response
Serum HCV-RNA was quantified at week 4 and week 12 to evaluate rapid virological response (RVR) and complete virological response (EVR), respectively. Similarly, we analyzed viral clearance at the end of the treatment (EOT) and 12 weeks after the EOT (SVR12). In order to evaluate the decline of serum HCV-RNA during the initial treatment phase, we also quantified the serum HCV-RNA on the 1st day and the 7th day after treatment was initiated. Quantification was performed using the COBAS® TaqMan® HCV Auto Assay System (Roche, USA; lower limit of quantification, 1.2 log10 IU/ml).

Statistical Analysis
The Pearson’s chi-square test or the Fisher’s exact test was used to compare categorical variables. For comparisons of continuous variables, the Wilcoxon rank-sum test and the Student t test were applied. All p values were 2-sided, and p value of <0.05 was considered statistically significant. All statistical analyses were performed using JMP version 9.0 software (SAS Institute Inc., Cary, N.C., USA).

Results
Alteration of Serum HCV-RNA during Treatment and Response to Prior Antiviral Therapy
Among the 46 patients treated with PEG-IFN/RBV/SMV, 20 were naïve cases, 12 were relapsers and 14 were NRs to prior dual therapy with PEG-IFN/RBV. Naïve cases and prior relapsers received treatment for 24 weeks (SMV12/PR12). Among 14 prior NRs, 2 patients who showed EVR (undetectable HCV-RNA at week 12) during PEG-IFN/RBV/SMV therapy received treatment with SMV12/PR36, while 7 NRs with EVR received SMV12/PR12 treatment. Five patients did not achieve serum HCV-RNA level <1.2 log10 IU/ml between the 12th week and the 24th week, and therapy was therefore discontinued before the completion of 24th week. Figure 1 illustrates the changes in serum HCV-RNA levels during and after treatment. We evaluated undetectable serum HCV-RNA 12 weeks after the completion of treatment (SVR12) in 42 patients, and of these, 30 achieved SVR12. Among 20 naïve cases, 1 patient showed EOT but did not achieve SVR12 (relapse) after the completion of triple therapy, and 1 patient showed breakthrough (BT) in the 20th week, although the HCV-RNA level was below the lower limit of quantification. Among 12 patients who showed relapse after the prior treatment, one relapsed again after triple therapy with PEG-IFN/RBV/SMV. Conversely, re-
lapse after triple therapy was observed in 4 of 14 NRs for prior treatment, and serum HCV-RNA did not reach the undetectable level (NR) in 5 patients.

Figure 2 shows the proportion of patients who achieved a defined virological response at each stage of the PEG-IFN/RBV/SMV treatment. Virological responses after initiation of treatment were defined as follows: achievement of HCV-RNA decline ≥2 log<sub>10</sub> IU/ml from baseline (2 log drop on day 1), HCV-RNA level <2 log<sub>10</sub> IU/ml on day 7 (<2 log on day 7), undetectable serum HCV-RNA at week 4 (RVR), undetectable serum HCV-RNA at week 12 (EVR) and undetectable serum HCV-RNA at the EOT. Although not statistically significant, prior NRs had a lower achievement rate than naïve cases and prior relapsers for 2 log drop on day 1, <2 log on day 7 and RVR, suggesting that the decline of serum HCV-RNA was slower in prior NRs than in naïve cases and prior relapsers. In addition, EVR, EOT and SVR12 rates were significantly lower in prior NRs than in naïve cases and prior relapsers. All naïve cases, all relapsers and 63% of NRs achieved EVR (p = 0.0020). In all, 94% of naïve cases, all relapsers and 64% of NRs achieved EOT (p = 0.0151). Finally, 88% of naïve cases, 92% of relapsers and 35% of NRs achieved SVR12 (p = 0.0014).

Pretreatment Conditions and Response to PEG-IFN/RBV/SMV Treatment

We analyzed the association between background conditions and response after PEG-IFN/RBV/SMV treatment. Among the background factors, IL-28B genotype was associated with viral response to PEG-IFN/RBV/SMV treatment in all phases of treatment, and T/T genotype was associated with good antiviral response (fig. 3a). Of special importance, significant associations were observed for EVR and SVR12, where 95% of T/T and 64% of T/G cases achieved EVR (p = 0.0367; fig. 3a), and 85% of T/T and 45% of T/G cases showed SVR12 (p = 0.0377; fig. 3a and table 1). On the other hand, no associations were observed between the presence of core 70 mutations and antiviral response (fig. 3b).

Among the 42 patients who were evaluated for SVR12, 31 were analyzed for IL-28B genotype. Twenty were of genotype T/T and 11 were of T/G genotype. Among the 20 patients with T/T genotype, 17 achieved SVR12 (85%), 2 showed relapse (10%) and 1 was considered as NR (5%) for triple therapy. On the other hand, among the 11 patients carrying T/G genotype, 5 (45%), 2 (18%) and 4 patients (36%) showed SVR12, BT/relapse and NR, respectively (p = 0.0437; fig. 3c).
Fig. 2. Percentage of the patients who achieved a defined virological response at each stage of the PEG-IFN/RBV/SMV treatment: 2 log drop on day 1, decrease of serum HCV-RNA is $2 \log_{10}$ IU/ml or greater from baseline on day 1 of the treatment; <2 on day 7, serum HCV-RNA level <2 $\log_{10}$ IU/ml; RVR, undetectable serum HCV-RNA at week 4; EVR, undetectable serum HCV-RNA at week 12; EOT, undetectable serum HCV-RNA at the EOT; SVR12, undetectable serum HCV-RNA 12 weeks after the EOT. p values are calculated by the Pearson’s chi-square test.

![Diagram](image1)

Fig. 3. The association between IL-28B genotype, presence of HCV with core 70 mutation and antiviral response of the PEG-IFN/RBV/SMV. Achievement rate in each phase of treatment is shown in the context of IL-28B genotype (a) and presence or absence of HCV core 70 mutation (b). p values are calculated by the Fisher’s exact test. Association between IL-28B genotype and responses to PEG-IFN/RBV/SMV treatment at 12 weeks after the EOT are shown (c). Among 20 patients with T/T genotype, 17 achieved SVR12. 2 showed BT/relapse and 1 was NR. On the other hand, among the 11 patients carrying T/G genotype, 5, 2 and 4 patients showed SVR12, BT/relapse and NR, respectively. p values are calculated by the Pearson’s chi-square test.
Figure 4 represents the association between response to prior treatment with PEG-IFN/RBV and achievement of SVR12 after triple therapy. Among 16 naïve cases, 14 had SVR12 (88%). Similarly, 11 of 12 prior relapsers achieved SVR12 (92%). On the other hand, only 5 showed SVR12 (36%) among 15 prior NRs (p = 0.0036; fig. 4). We also conducted multivariate analysis using *IL-28B* genotype and response to prior treatment as co-variables. Response to prior treatment was identified as an independent factor for achieving SVR12 after triple therapy (naïve or relapser vs. NR, p = 0.0005, OR 37.4, 95% CI 4.30–875.7; table 1).

Table 1. Pretreatment conditions that are associated with achievement of SVR at 12 weeks post treatment

<table>
<thead>
<tr>
<th>Pretreatment Conditions</th>
<th>SVR12 (+) (n = 30)</th>
<th>SVR12 (–) (n = 12)</th>
<th>p value</th>
<th>OR (95% CI)</th>
<th>p value</th>
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</thead>
<tbody>
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<tr>
<td></td>
<td>64 (60–68)</td>
<td>57 (51–63)</td>
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<tr>
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<td>Male/female</td>
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<td></td>
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<tr>
<td></td>
<td>12/18</td>
<td>5/7</td>
<td>1.0000</td>
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<tr>
<td><em>IL-28B</em>1</td>
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<tr>
<td></td>
<td>17/5</td>
<td>3/6</td>
<td>0.0377</td>
<td>2.72 (0.25–30.0)</td>
<td>0.3951</td>
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<tr>
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<td>5/3</td>
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<tr>
<td></td>
<td>25/5</td>
<td>3/9</td>
<td>0.0006</td>
<td>37.4 (4.30–875.7)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

1 Genotype is determined at rs8099917.
2 Presence (mutant) or absence (wild) of HCV with the mutant core 70 mutation.
3 Response to prior treatment with PEG-INF and RBV. Naïve, a patient without prior treatment. Relapser, a patient who showed undetectable HCV-RNA at the end of the treatment but detectable HCV-RNA during follow-up. NR, a patient who never showed undetectable HCV-RNA. p values <0.05 are shown in bold.

**EVR and Achievement of SVR12 in PEG-IFN/RBV/SMV Treatment**

We also analyzed the association between an early antiviral response for PEG-IFN/RBV/SMV triple therapy and achievement of SVR12 (fig. 5). Reduction of 2 log on day 1, <2 log on day 7, RVR, EVR and EOT were associated with SVR12 (this was statistically significant for <2 log on day 7, RVR, EVR and EOT with p values 0.0050, 0.0002, 0.0009 and 0.0002, respectively). All patients who failed to achieve EVR and EOT resulted in relapse, BT or NR, after PEG-IFN/RBV/SMV triple therapy. Among 21 patients who showed <2 log on day 7, 19 achieved SVR12 (positive predictive value (PPV 91%)). Ten patients failed to achieve SVR12 among 19 patients that did not demonstrate <2 log on day 7 (negative predictive value (NPV 53%)). Similarly, among 29 patients who showed RVR, 16
achieved SVR12 (PPV 90%), and 9 failed to achieve SVR12 among 13 patients without RVR (NPV 69%). The 16 naïve cases and the 12 prior relapers could be evaluated for SVR12. Of these, 3 patients failed to achieve SVR12. Notably, in 2 of 3 naïve/prior relapers who did not show SVR12 after triple therapy, HCV-RNA was detectable at week 4, although it was below the lower limit of quantification. On the other hand, among 5 patients who were NRs to prior treatment and achieved SVR12 after triple therapy, all showed undetectable HCV-RNA at week 4, although 2 of 5 patients received SMV12/PR36.

**Discussion**

Recently, IFN-free regimens for CHC using DAAs have been shown to achieve high SVR rates, even for patients who did not respond to IFN-based therapy [13]. On the other hand, there are still concerns about IFN-free regimens, and emergence of mutated HCV resistant to DAAs could affect the efficacy of future antiviral treatments [14, 15]. Furthermore, in contrast to IFN-based therapy, the suppressive effect of DAAs on HCC development is still unclear [5]. Therefore, for treatment of CHC with genotype 1b and high viral load, triple therapy using a second-generation protease inhibitor, PEG-IFN, and RBV should still be considered for naïve cases, as well as for re-treatment cases without a history of protease inhibitor use. This approach is supported by the most recent guidelines for the management of HCV infection proposed by the Japan Society of Hepatology (http://www.jsh.or.jp/medical/guidelines/jsh_guidlines/hepatitis_c). However, failure of a triple regimen could also lead to the emergence of resistant viruses for NS3/4A protease inhibitors, which is mainly attributable to the amino acid substitution at D168 [14, 16]. From this point of view, predicting relapse in the early phase of treatment is important so the length of treatment can be extended such as SMV12/PR36 regimen. In this study, we demonstrated that EVR is a predictive factor for the achievement of SVR12, even in antiviral therapy with PEG-IFN/RBV/SMV.

In concordance with earlier studies, our data also show that a genotype of IL-28B and responses to prior treatment are significantly associated with the achievement of SVR12 following triple regimen therapy [8]. Response to prior treatment is identified as a particularly important independent factor for SVR12. It is also well documented that response to prior IFN-based treatment reflects the antiviral effect of IFN that is critical for achieving SVR, even for patients treated with PEG-IFN/RBV/SMV [9, 11]. Our data also show that SVR12 rates are high in naïve cases and prior relapers, reaching 88 and 92%, respectively.

On the other hand, 2 naïve cases and 1 relaper failed to achieve SVR12. Two of the 3 patients who were naïve...
or prior relapers, and failed to achieve SVR12, revealed detectable HCV-RNA at week 4, although the level was below the lower limit of quantification. On the other hand, 5 prior NRs achieved SVR12 following triple therapy; all of them showed RVR. Therefore, we further analyzed the viral response of each phase of treatment in the context of achieving SVR12. We found that, in addition to EVR and EOT, the degree of HCV decline on day 7 and the rate of undetectable HCV at week 4 were both significantly associated with subsequent achievement of SVR12 (PPV and NPV for SVR12 were 91 and 53% for achievement of <2 log on day 7, and 90 and 69% for RVR, respectively). It has been previously reported that the decline of serum HCV-RNA at treatment week 4 was a good marker of SVR in patients with HCV genotype 1/high viral load, who received treatment with response-guided PEG-IFN plus RBV combination therapy [17]. This evidence suggests that EVR is a surrogate marker of the antiviral effect of IFN, and this could affect antiviral response even in treatment with PRG-IFN/RBV/SMV.

In this report, we showed that rapid decline of HCV and undetectable HCV-RNA at week 4 of treatment, should be a surrogate marker for predicting SVR12 in triple therapy using REG-IFN/RBV/SMV in both naïve/prior or relaper and in NRs. From this point of view, extension of the treatment period should be considered for patients with detectable serum HCV-RNA at week 4, even in naïve cases and prior relapers to PEG-IFN/RBV.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

References

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References

2 Fried MW, Buti M, Dore GJ, Flisiak R, Fenci P, Jacobson I, Marcellin P, Manns M, Niki-<ref>tin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smert G, Beumont-Mauvel M: Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naive genotype 1 hepatitis C: the randomized PILLAR study. Hepatology 2013;58:1918–1929.</ref> 3 Forns X, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, Horban A, Brown A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, Scott J, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauvel M: Simeprevir with peginterferon and ribavirin in treatment-naive patients who received treatment with response-guided PEG-IFN plus RBV combination therapy; all of them showed RVR. Therefore, we further analyzed the viral response of each phase of treatment in the context of achieving SVR12. We found that, in addition to EVR and EOT, the degree of HCV decline on day 7 and the rate of undetectable HCV at week 4 were both significantly associated with subsequent achievement of SVR12 (PPV and NPV for SVR12 were 91 and 53% for achievement of <2 log on day 7, and 90 and 69% for RVR, respectively). It has been previously reported that the decline of serum HCV-RNA at treatment week 4 was a good marker of SVR in patients with HCV genotype 1/high viral load, who received treatment with response-guided PEG-IFN plus RBV combination therapy [17]. This evidence suggests that EVR is a surrogate marker of the antiviral effect of IFN, and this could affect antiviral response even in treatment with PRG-IFN/RBV/SMV.

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