Therapeutic drug monitoring of telaprevir in chronic hepatitis C patients receiving telaprevir-based triple therapy is useful for predicting virological response

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Objectives: This prospective, pharmacokinetic study was done to investigate the impact of telaprevir plasma trough concentration (C\text{trough}) in the early stage of treatment on the response to telaprevir-based triple therapy for chronic hepatitis C patients.

Methods: Participants were 70 chronic hepatitis C patients infected with genotype 1. All patients received 12 week triple therapy that included telaprevir (2250 mg/day), pegylated interferon-\textalpha\textsubscript{2b} (pegylated-IFN\textalpha\textsubscript{2b}) (60 – 150 μg/week) and ribavirin (600 – 1000 mg/day) followed by a 12 week dual therapy that included pegylated-IFN\textalpha\textsubscript{2b} and ribavirin. Plasma telaprevir C\text{trough} was determined by a validated assay using HPLC at days 3, 7 and 14. The study was registered as a clinical trial on the University Hospital Medical Information Network (ID 000009656).

Results: The rates of undetectable hepatitis C virus RNA at week 4 (rapid virological response (RVR)) and at 24 weeks after therapy (sustained virological response (SVR)) were 71.4% and 82.9%, respectively. Of the patients with RVR, 90% achieved SVR. The mean telaprevir C\text{trough} levels at days 3, 7 and 14 of SVR patients (2.748, 2.733 and 2.999 μg/mL, respectively) were significantly higher than those of non-SVR patients (1.616, 1.788 and 2.314 μg/mL, respectively) (all \(P<0.05\)). Multiple logistic regression analysis of possible predictors of SVR extracted higher telaprevir C\text{trough} at day 3 (OR 1.012 by 0.001 μg/mL, \(P<0.0001\)) and interleukin 28B (rs8099917) TT allele (OR 6.16 versus non-TT alleles, \(P<0.0001\)).

Conclusions: Therapeutic drug monitoring of telaprevir in the early stage of treatment is useful in clinical practice for predicting the virological response of patients receiving telaprevir-based triple therapy.

Keywords: plasma trough concentrations, pegylated interferon-\textalpha, ribavirin

Introduction

Chronic hepatitis C virus (HCV) infection affects ~170 million people and often causes cirrhosis and hepatocellular carcinoma (HCC).\textsuperscript{1, 2} The ultimate goal of treatment for chronic hepatitis C is sustained virological response (SVR), which is defined as undetectable HCV RNA in serum 6 months after the termination of treatment. A 48 week combination of pegylated interferon-\textalpha (pegylated-IFN\textalpha) plus ribavirin is successful for only ~45% of the ‘difficult-to-treat’ chronic hepatitis C patients infected with HCV genotype 1.\textsuperscript{3 – 6}

Analysis of serum HCV dynamics has been shown to be useful for predicting the efficacy of antiviral treatment for chronic hepatitis C. Serum HCV RNA decline in the early stage of treatment (4 – 12 weeks after the start of treatment) is associated with the likelihood of achieving an SVR.\textsuperscript{3 – 6} Rapid virological response (RVR; undetectable HCV RNA at week 4) and early virological response (EVR; undetectable HCV RNA at week 12) can provide guidance as to the likelihood of achieving SVR.\textsuperscript{3 – 6}

Both pegylated-IFN\textalpha and ribavirin are indirect antiviral agents. They do not target a specific HCV protein or nucleic acid. In the USA, Canada, the European Union and Japan, telaprevir (VX950), an HCV non-structural 3/4A protease inhibitor, has recently been approved for the treatment of chronic hepatitis C genotype 1 and represents a new class of small molecules that are direct-acting antiviral agents (DAAs) that interfere with HCV replication.\textsuperscript{7 – 11} Telaprevir-based triple therapy combined with pegylated-IFN\textalpha and ribavirin has resulted in an improved SVR rate, when compared with pegylated-IFN\textalpha monotherapy and pegylated-IFN\textalpha plus ribavirin dual therapy.\textsuperscript{12, 13} This is because telaprevir can produce a very rapid decline of the serum HCV RNA level immediately after the start of administration.\textsuperscript{14 – 18} A steady-state after multiple doses of...
orally administered telaprevir is reached after 3–7 days of administration.7–10 Therefore, we hypothesized that the telaprevir plasma trough concentration (C_{trough}) in the early stages of treatment (days 3–14) would have a great impact on the virological response of patients with HCV genotype 1 chronic hepatitis C. The aim of this prospective pharmacokinetic study was to investigate the relationship between the telaprevir C_{trough}, and the virological response of these patients.

Patients and methods

Study participants

This prospective study consisted of 70 Japanese patients infected with HCV genotype 1b, including 20 (28.6%) treatment-naive patients, 29 (41.4%) with prior relapse and 21 (30.0%) with prior non-response. Relapse was defined as undetectable HCV RNA during and at the end of treatment, but with HCV RNA positivity returning later. Non-response was defined as detectable HCV RNA for >24 weeks. All 50 prior relapse and non-response patients had a history of a 48 week pegylated-IFNα plus ribavirin combination treatment.

Telaprevir is contraindicated when combined with drugs that are highly dependent on cytochrome P450, family 3, subfamily A (CYP3A) for clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events.10,11 Also, telaprevir is contraindicated when combined with drugs that strongly induce CYP3A and thus may lead to lower exposure and loss of telaprevir efficacy.12 Based on the label instructions for each product, the drugs of patients taking contraindicated concomitant medications were switched to alternative drugs ≥3 months before the initiation of treatment.

All patients were recruited at Kyushu University Hospital. Exclusion criteria were as follows: positivity for antibody to HIV or positivity for hepatitis B surface antigen; clinical or biochemical evidence of hepatic decompensation; other causes of liver disease; excessive active alcohol consumption (>40 g/day of ethanol) or drug abuse; suspected HCC or active cancer at entry; chronic renal failure or estimated glomerular filtration rate of ≤50 mL/min; very poorly controlled heart diseases, pulmonary disorders, thyroid diseases or diabetes; depression or its history; history of a suicide attempt; pregnancy of either partner or planned during the study period; or treatment with antiviral or immunosuppressive agents prior to enrolment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital. Informed consent was obtained from each patient before enrolment. The study was registered as a clinical trial on the University Hospital Medical Information Network (ID 000009656).

Clinical assessment

Clinical parameters included serum albumin, creatinine, total bilirubin, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, estimated glomerular filtration rate, white blood cell count, haemoglobin, platelet count and serum HCV markers. Blood samples were taken at baseline, days 3 and 7, and every week thereafter to week 24 during the treatment period. All parameters were measured at our hospital by standard laboratory techniques. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). Liver biopsy at entry for 65 (92.9%) of the 70 patients was done by two or more experienced hepatologists. For each specimen, the stage of fibrosis (F0–4) and the grade of activity (A0–3) were established according to METAVIR score.19

Determination of HCV markers

The baseline and follow-up tests for HCV viraemia were done by real-time PCR assay (COBAS® AmpliPrep/COBAS® TaqMan® HCV assay; Roche Molecular Systems, Inc., Branchburg, NJ, USA), with a lower limit of quantification of 15 IU/mL and an upper limit of quantification of 6.9 × 10⁷ IU/mL (1.2–7.8 log IU/mL referred to log10 U/mL). HCV genotype and the core amino acid substitution at position 70 of the HCV gene were determined for each patient before treatment. HCV genotype was determined by sequence determination in the 5′-non-structural region of the HCV genome followed by phylogenetic analysis.20 Amo acid substitution at position 70 of the core region was analysed by direct sequencing, as reported previously.21

Genetic testing

Human genomic DNA was extracted from the peripheral blood of each patient. Genotyping by single nucleotide polymorphism (SNP) of the interleukin 28B (IL28B) (rs8099917) gene was done using the TaqMan® Allelic Discrimination Demonstration Kit (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA, USA). Patien ts were genotyped as TT, TG or GG at the polymorphic site. Genotyping by SNP of the inosine triphosphate pyrophosphatase (ITPA) (rs1127354) gene was done using the TaqMan® Allelic Discrimination Demonstration Kit. Patients were genotyped as CC, CA or AA at the polymorphic site.22–23

Antiviral treatment

All patients received 12 week triple therapy that included telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), pegylated-IFNα2b (PEG-Intron; MSD, Tokyo, Japan) and ribavirin (Rebetol; MSD) followed by a 12 week dual therapy that included pegylated-IFNα2b and ribavirin. Telaprevir (2250 mg/day) was administered orally three times a day, 750 mg after each meal at 8 h intervals. Pegylated-IFNα2b was injected subcutaneously once a week at a dose of 1.5 μg/kg. Ribavirin was given orally at a daily dose of 600–1000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60–80 kg and 1000 mg for those weighing >80 kg). Dose reduction from the starting dose (1.5 μg/kg/week) of pegylated-IFNα2b was done in a two-step process: first to 1 μg/kg/week, then to 0.5 μg/kg/week, if needed. If haemoglobin <120 g/L was observed during treatment, the first dose reduction of ribavirin was by 200 mg/day and the second dose reduction was by an additional 200 mg/day when haemoglobin <100 g/L was observed. The above protocol is that approved by the Japanese Ministry of Health, Labour and Welfare.24 Telaprevir dose was reduced to 1500 mg/day (750 mg twice a day at a 12 h interval after meals) if a 1.5–2.0 times increase from baseline serum creatinine was observed. When serum creatinine was elevated to >2.0 times the baseline level, telaprevir was discontinued. If marked anorexia developed, the telaprevir dose could be reduced to 1500 mg/day. When grade 3 rash (≥50% of the body surface or with the appearance of substantial systemic signs of symptoms) developed, telaprevir was discontinued. However, the patients continued to receive pegylated-IFNα2b and ribavirin in all of these situations. All treatment was discontinued for patients with <2 log₁₀ HCV RNA decline from baseline to week 12.25 The triple therapy was discontinued if the haemoglobin, white blood cell count or platelet count fell below 80 g/L, 1 × 10⁹/L or 50 × 10⁹/L, respectively.26

Efficacy of treatment

Successful treatment corresponded to SVR, defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. EVR during the first 12 weeks of treatment was categorized as follows: RVR, undetectable HCV RNA at week 4; and complete EVR (cEVR), detectable HCV RNA at week 4, but undetectable at week 12. End-of-treatment response was defined as undetectable HCV RNA at the end of treatment. Relapse was defined as an end-of-treatment response, but non-SVR.

Assessment of drug adherence

The weekly dose of pegylated-IFNα2b and the daily doses of ribavirin were recorded by patients in an electronic dosing diary. Adherence to the

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prescribed dosage of each drug was defined as the total dosage of the drug taken by the patient divided by the expected total drug dosage, with the expected total drug dose based on the actual treatment duration.

**Determination of telaprevir C_{trough}**

Blood samples, collected early in the morning before the first daily dose of telaprevir, were drawn prior to each drug administration for determination of the telaprevir C_{trough}, and the sampling window time from the last dose was calculated for each patient. Serial pharmacokinetic assessment of telaprevir C_{trough} was done on days 3, 7 and 14 after the first administration of telaprevir. Samples from the vein (~2.0 mL) were collected in tubes containing K3 EDTA. Each tube was immediately cooled in an ice water bucket, followed by centrifugation within 1 min after collection at ~1500 g for 10 min at 4 °C. After 1 mL of the plasma (in duplicate) was mixed well with 50 μL of 10% formic acid to acidify the plasma and stabilize the telaprevir, the mixture was frozen on dry ice followed by storage at −20 °C or below in a freezer.

The plasma telaprevir S-isomer (VX950) and R-isomer levels were simultaneously determined using a validated assay based on HPLC with mass spectrometry at a commercial laboratory (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The assay was validated over a telaprevir concentration range of 0.1–10 μg/mL for sensitivity, linearity, reproducibility, stability and recovery rate. The intra- and interassay precision (coefficient of variation %) met the criterion of <15% and accuracy (relative error to the nominal concentration) results ranged within ±15%.

**Statistical analysis**

Statistical analyses were performed using the SAS system, version 9.1.3 (SAS Institute, Cary, NC, USA). Continuous data are expressed as the mean (±SD) and compared using a t-test or Wilcoxon rank-sum test. Categorical variables are expressed as frequencies and percentages and compared using the χ² test, Fisher's exact test or Mann–Whitney U-test. Telaprevir C_{trough}, is expressed as the mean (±SD). An area under the receiver operating characteristic curve (AUROC) analysis was conducted to evaluate the relationship between the telaprevir C_{trough} and SVR. The cut-off values were selected from the receiver operating characteristic (ROC) curve to maximize the total sensitivity and specificity. We compared the AUROC of the two ROC curves using the method described by DeLong et al.24 Univariate analysis was initially performed and variables with a significant association to SVR (P < 0.2) were subsequently evaluated by stepwise logistic regression analysis that included all available variables in the database. Hosmer–Lemeshow lack-of-fit tests were performed to assess the fit of our final multivariate models. ORs with their 95% CIs were determined. A P value <0.05 was regarded as statistically significant in all analyses.

**Results**

**Patient characteristics**

The patient characteristics are summarized in Table 1. SVR patients had a significantly higher mean platelet count and serum albumin level than non-SVR patients. The IL28B SNP (rs8099917) TT allele was more frequent in SVR patients than in non-SVR patients. Non-TT (TG/GG) alleles were more frequent in non-SVR patients than in SVR patients. Prior relapse was significantly more frequent in SVR patients than in non-SVR patients. Prior non-response was significantly more frequent in non-SVR patients than in SVR patients.

None of the patients experienced severe anaemia before week 2 and only two discontinued telaprevir due to severe anaemia after week 2 (at weeks 4 and 10, respectively). Five others (7.1%) needed a dose reduction of telaprevir (from 2250 to 1500 mg/day) due to severe anorexia after week 2 and two of them completely stopped telaprevir due to progressively severe anorexia (at weeks 6 and 12, respectively). Of the 70 patients studied, only these 7 were not able to complete the full telaprevir regimen. The remaining 63 patients continued to receive pegylated-IFNα2b and ribavirin. However, all 70 patients completed the first 2 weeks of the telaprevir regimen.

**Virological outcome and response**

Virological outcome and response during treatment were analysed on an intention-to-treat basis. RVR, cEVR, end-of-treatment response and SVR were found for 50 (71.4%), 18 (25.7%), 69 (98.6%) and 58 (82.9%) of the 70 studied patients, respectively: 11 patients relapsed (15.7%). Patients with RVR had a higher rate of SVR (90.0%, 45 of 50) than cEVR (72.2%, 13 of 18), but the difference did not reach significance (P=0.067). The mean serum HCV RNA level (±SD) at day 3 was significantly lower for RVR patients (2.2±0.8 log IU/mL) than for non-RVR patients (3.0±0.5 log IU/mL) (P < 0.0001), whereas no significant difference in the level at day 3 was found between SVR (2.4±0.8 log IU/mL) and non-SVR (2.6±0.8 log IU/mL) patients (P=0.429).

**Changes in telaprevir C_{trough} by virological response**

Table 2 shows the plasma telaprevir C_{trough} levels at each test point. The mean (±SD) sampling window time of a total of 210 plasma samples obtained from the 70 patients was 10.2±1.1 h after the last dose. No significant differences in the mean window times were found between SVR and non-SVR patients. Stratification by virological outcome revealed differences in the telaprevir C_{trough} levels of SVR and non-SVR patients (Figure 1). At each point (days 3, 7 and 14), the mean plasma telaprevir C_{trough} level of the SVR patients (2.748, 2.733 and 2.999 μg/mL, respectively) was significantly higher than that of non-SVR patients (1.616, 1.788 and 2.314 μg/mL, respectively) (P<0.0001, P=0.0004 and P=0.0380, respectively).

Stratification by EVR revealed differences in the telaprevir C_{trough} levels of RVR (n=50) and non-RVR (n=20) patients. At each point (days 3, 7 and 14), the mean plasma telaprevir C_{trough} level of RVR patients (2.661, 2.580 and 3.064 μg/mL, respectively) was higher than that of non-RVR patients (2.429, 2.541 and 2.800 μg/mL, respectively), but without statistical significance.

The rate of SVR of pre-cirrhosis (F3) and cirrhosis (F4) patients (78.6%, 22 of 28) was lower than that of patients with mild liver fibrosis (F0–2) (83.8%, 31 of 37), but the difference was not significant (P=0.591). We conducted an additional analysis of the telaprevir C_{trough} of the F3 and F4 patients, classified by SVR (Figure 2). At the day 3, 7 and 14 test points, the mean plasma telaprevir C_{trough} level of the SVR patients (2.616, 2.609 and 3.063 μg/mL, respectively) was higher than that of non-SVR patients (1.492, 1.701 and 2.099 μg/mL, respectively). The high telaprevir C_{trough} in the early stage (days 3, 7 and 14) was related to SVR of the patients with progressive liver fibrosis (P=0.0021, P=0.0043 and P=0.0334, respectively).

**Changes in telaprevir C_{trough} by baseline factors and drug adherence**

Univariate analyses found no significant differences in plasma telaprevir C_{trough} between men and women, IL28B TT and non-TT alleles, or core wild or mutated HCV. Moreover, we did not find any significant differences in telaprevir C_{trough} levels for patient
<table>
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<tr>
<th>Variable</th>
<th>SVR (n = 58)</th>
<th>Non-SVR (n = 12)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>30 (51.7)</td>
<td>5 (41.7)</td>
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<tr>
<td>Age (years)</td>
<td>58.8 ± 10.4</td>
<td>63.4 ± 6.8</td>
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<td>Body mass index (kg/m²)</td>
<td>23.5 ± 3.2</td>
<td>22.9 ± 3.5</td>
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<td>Baseline HCV RNA (log IU/mL)</td>
<td>6.4 ± 0.6</td>
<td>6.5 ± 0.5</td>
<td>0.903</td>
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<td>HCV core amino acid substitution at position 70, wild/mutation, n/n</td>
<td>32/26</td>
<td>5/7</td>
<td>0.393</td>
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<td>White blood cell count (×10⁶/L)</td>
<td>5117 ± 1451</td>
<td>4257 ± 985</td>
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<td>Haemoglobin level (g/L)</td>
<td>142 ± 16.8</td>
<td>130 ± 11.9</td>
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<td>Platelet count (×10⁹/L)</td>
<td>161 ± 49</td>
<td>128 ± 65</td>
<td>0.009</td>
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<td>Serum albumin (g/L)</td>
<td>41 ± 3</td>
<td>38 ± 3</td>
<td>0.007</td>
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<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>54.8 ± 35.9</td>
<td>66.4 ± 30.7</td>
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<td>Alanine aminotransferase (IU/L)</td>
<td>55.3 ± 34.8</td>
<td>55.9 ± 28.1</td>
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<tr>
<td>γ-Glutamyl transpeptidase (IU/L)</td>
<td>72.0 ± 109.0</td>
<td>59.7 ± 41.8</td>
<td>0.564</td>
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<td>Estimated glomerular filtration rate (mL/min/1.73 m²)</td>
<td>105.3 ± 16.2</td>
<td>105.9 ± 12.4</td>
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<td>Liver histology</td>
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<td>5/7</td>
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<td>Previous treatment outcome, n (%)</td>
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<td>treatment naive</td>
<td>16 (27.6)</td>
<td>4 (33.3)</td>
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<td>prior relapse</td>
<td>28 (48.3)</td>
<td>1 (8.3)</td>
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</tr>
<tr>
<td>prior non-response</td>
<td>14 (24.1)</td>
<td>7 (58.3)</td>
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<td>IL28B SNP (rs8099917), n (%)</td>
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<td>TT</td>
<td>36 (62.1)</td>
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<td>TG/GG</td>
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<td>7 (58.3)</td>
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<td>ITPA SNP (rs1127354), n (%)</td>
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<tr>
<td>CC</td>
<td>45 (77.6)</td>
<td>7 (58.3)</td>
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<td>CA/AA</td>
<td>13 (22.4)</td>
<td>5 (41.7)</td>
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<td>Adherence to pegylated-IFNa2b dosage ≥80% and ribavirin dosage ≥80%, n (%)</td>
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<td>Adherence to pegylated-IFNa2b dosage ≥80%, n (%)</td>
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<tr>
<td>Adherence to ribavirin dosage ≥80%, n (%)</td>
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<td>Discontinuation of pegylated-IFNa2b and ribavirin, n (%)</td>
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<tr>
<td>Discontinuation of telaprevir, n (%)</td>
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<td>Discontinuation of triple therapy, n (%)</td>
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<td>Telaprevir dose reduction, n (%)</td>
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<tr>
<td>RVR, n (%)</td>
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<tr>
<td>cEVR, n (%)</td>
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All patients were infected with HCV genotype 1b. Continuous variables are expressed as mean ± SD. Relapse was defined as undetectable HCV RNA during and at the end of treatment, with HCV RNA positivity returning later. Non-response was defined as detectable HCV RNA for >24 weeks. All 50 with prior relapse or non-response had a history of pegylated-IFNa plus ribavirin combination treatment. <sup>a</sup>P value for comparisons between the SVR and non-SVR groups.
age, BMI, platelet count, liver fibrosis stage, prior treatment response, ITPA allele or adherence to pegylated-IFNa2b or ribavirin.

**ROC curve analysis of telaprevir C\text{trough} for predicting an SVR**

Table 3 shows the results of ROC curve analysis conducted to evaluate the ability of plasma telaprevir C\text{trough} to predict SVR. The AUROC value at day 3 was the highest and differed significantly from those at days 7 (P = 0.0001) and 14 (P < 0.0001). The optimal telaprevir C\text{trough} threshold was 1.924 \(\mu\text{g/mL} at day 3, which suggests that it is the best predictor of SVR.

**Variables correlated with SVR**

In order to identify variables significantly associated with achieving an SVR, variables with P < 0.2 in univariate analysis in Table 1 (age, white blood cell count, platelet count, serum albumin, aspartate aminotransferase, previous treatment outcome, IL28B SNP, ITPA SNP, discontinuation of pegylated-IFNa2b and ribavirin, discontinuation of triple therapy, RVR and cEVR) and telaprevir C\text{trough} at day 3 were evaluated by multivariate logistic regression. This analysis of possible predictors of SVR extracted higher telaprevir C\text{trough} at day 3 (OR 1.012 by 0.001 \(\mu\text{g/mL}, 95\% \text{CI} 1.001 – 1.060, P < 0.0001) and IL28B (rs8099917) TT allele (OR 6.16 versus non-TT alleles, 95\% CI 2.54 – 17.9, P < 0.0001).

**Discussion**

This study shows the usefulness of telaprevir concentration monitoring for patients receiving telaprevir-based triple therapy and its utility for predicting the virological response of patients with HCV genotype 1b. After a single dose of telaprevir, the mean half-life is \(\approx 4\) h. At steady-state, the half-life is \(\approx 9 – 11\) h. When 750 mg of telaprevir is administered three times a day at 8 h intervals, a steady-state is reached from 3 to 7 days after the start of administration.\(^7\)–\(^10\) Our findings showed significant differences in the plasma telaprevir C\text{trough} levels of SVR and non-SVR patients in the early stage (days 3 - 14) of triple therapy, suggesting that early telaprevir monitoring would help the tailoring of treatment duration and that it could be useful as an early predictor of treatment failure. To date, no studies have assessed the relative role played by telaprevir C\text{trough} on the virological efficacy of telaprevir-based triple therapy. Our study showed that higher telaprevir C\text{trough} in the early stage is related to both RVR and SVR. We also showed that a significant decline of the serum HCV RNA level at day 3 is related to RVR, which leads to SVR. This phenomenon is a virological feature that clearly shows the rapid action of the DAA telaprevir.

Two patients stopped telaprevir due to adverse effects in this study. To avoid telaprevir-resistant variants of HCV, telaprevir dose reduction should not be permitted. This study included five patients who had the dose of telaprevir reduced due to adverse effects after week 2. In this study, 10\% (7 of 70) of the patients were unable to complete the telaprevir regimen. This must be taken into account when interpreting the results. This is because our study is not a sponsor-initiated clinical trial, but an investigator-initiated clinical research project. However, all of the 70 patients studied completed the full telaprevir regimen of the first 2 weeks. For this reason, we focused on the analysis of the association between telaprevir C\text{trough} in the early stage and on SVR.

Treatment of chronic hepatitis C has two goals. The first is sustained eradication of HCV. The second is to prevent progression...
to cirrhosis, HCC or decompensated liver disease. We previously showed a significant association between SVR and a lower rate of HCC in a prospective, multicentre study. However, a large cohort study (n = 497) in France has provided significant information for the management of DAA-based triple therapy for HCV-infected cirrhotic patients, showing that the safety profile is poor in a real-life setting. Therefore, treatment should be initiated promptly for patients with advanced fibrosis. We recruited 28 patients with progressive liver fibrosis (F3 and F4), of whom 22 achieved SVR and 6 had treatment failure. In this subgroup, we found a difference in the telaprevir Ctrough in the early stage of treatment related to virological response. These findings give us a tool for the early prediction of treatment, which will help prevent unnecessary exposure to the strong drugs used for patients with advanced fibrosis. Although our results are based on a limited number of patients, they indicate that drug monitoring during DAA treatment with the next generation of drugs will be helpful, even for patients with progressive liver fibrosis.

Gastrointestinal tract absorption probably influences telaprevir pharmacokinetics. Absorption through the gastrointestinal tract is influenced by the fat content of food. Compared with standard food ingestion (533 kcal, 189 kcal of fat), the telaprevir concentration decreased by 73%–83% when administered under fasting conditions. For our patients, telaprevir administration immediately after each meal was recommended, as instructed in the telaprevir prescription literature. Unfortunately, we did not record food consumption in terms of calories or constituents and thus cannot perform a more detailed analysis of the differences in the telaprevir Ctrough.

The IL28B gene-related SNP on chromosome 19 is the most important baseline predictor of SVR after treatment with pegylated-IFNα plus ribavirin for chronic hepatitis C. This study found the IL28B allele to be a significant, independent pretreatment factor for achieving SVR with this telaprevir-based regimen. Several classes of DAA are under development and it is expected that, when used in combination with pegylated-IFNα plus ribavirin, these new drugs will improve SVR and decrease the required duration of treatment. The IL28B allele has an influence on early viral kinetics, even during treatment with interferon-free DAA.

Table 3. ROC curve analysis to determine the plasma telaprevir Ctrough predictive of SVR

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.859</td>
<td>0.596</td>
<td>0.857</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.917</td>
<td>0.906</td>
<td>0.500</td>
</tr>
<tr>
<td>AUROC</td>
<td>0.910</td>
<td>0.828</td>
<td>0.691</td>
</tr>
<tr>
<td>Cut-off value (µg/mL)</td>
<td>1.924</td>
<td>2.496</td>
<td>1.840</td>
</tr>
<tr>
<td>P value</td>
<td>reference</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*P values were calculated in comparison with the day 3 value for AUROC.
regimens. Interferon-free regimens produce no difference within 6 days after the start of treatment, but a significant difference in HCV RNA reduction after day 7 has been documented for patients with IL28B SNP (rs12979860) CC versus the non-CC alleles. The host IL28B allele has also been shown to affect the spontaneous clearance of HCV infection. Thus, IL28B genotyping will continue to play an important role in determining the likelihood of anti-HCV treatment response.

A limitation of this study is that the sample size might provide inadequate statistical power to detect definitive differences in the telaprevir C\text{trough} between SVR and non-SVR. However, this was a prospective study and included consecutive data on the telaprevir C\text{trough} in the early stage of telaprevir-based triple therapy. To the best of our knowledge, ours is the first significant pharmacokinetic study of telaprevir C\text{trough}. Also, we studied only patients with HCV genotype 1b. Significant differences in virological response and drug mutations between HCV genotypes 1a and 1b have been reported for DAA-based treatment. We are uncertain whether our finding that higher telaprevir C\text{trough} can lead to the achievement of SVR applies to the response of other HCV genotypes. Further study will be required to clarify the importance of telaprevir C\text{trough} on treatment outcome. Our patients received a fixed 24 week triple therapy. Telaprevir has been approved since September 2011 for use in Japan only for the treatment of genotype 1 chronic hepatitis C with a high HCV RNA level ($\geq 5.0\log$ IU/mL). Also, the duration of triple therapy is 24 weeks, with all three agents for the first 12 weeks and then pegylated-IFN\text{a}-2b plus ribavirin dual therapy for the remaining 12 weeks. In contrast, in Europe, the USA and Canada, telaprevir must be administered with pegylated-IFN\text{a} and ribavirin for all patients for 12 weeks, followed by a response-guided regimen of either 12 or 36 additional weeks of pegylated-IFN\text{a} and ribavirin, depending on the early viral response. Differences in the duration of treatment after the end of telaprevir administration could influence relapse, but not end-of-treatment response. Therefore, our findings on efficacy as judged by the early-stage telaprevir C\text{trough} should hold, even for prolonged regimens of pegylated-IFN\text{a}-2b and ribavirin. To ensure compliance, each patient was instructed before and during treatment that telaprevir should be administered three times a day, after each meal at an 8 h interval. However, our data showed that $\sim 10$ h was the mean window time of the telaprevir C\text{trough}. This difference in timing must be considered when judging the findings. The difference may have occurred because the patients had difficulty adjusting to taking meals in the middle of the night. This is problematic in any investigator-initiated clinical research.

In conclusion, our study shows that the telaprevir C\text{trough} threshold at day 3 is highly predictive of SVR in patients undergoing a telaprevir-based treatment regimen. The results provide important new insights that will be useful for developing DAA-based regimens.

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**Transparency declarations**

None to declare.

**Author contributions**

The conception and design of the study was carried out by N. F., E. O., M. M. and J. H. The acquisition of data was done by N. F., E. O., M. M., K. T., H. O., K. E., M. S., Y. H. and F. M. The data were analysed by N. F., E. O. and M. M., and interpreted by all co-authors. All authors contributed to the drafting of the paper and its revision and are responsible for the intellectual content and the final approval of the version to be published.

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