Telaprevir and Ribavirin Interaction: Higher Ribavirin Levels Are Not Only Due to Renal Dysfunction during Triple Therapy

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A higher incidence of anemia has been observed during the treatment of hepatitis C virus genotype 1 (HCV-1) infection with pegylated alpha interferon (pegIFN-α), ribavirin, and telaprevir. We assessed the impacts that concomitant administration of telaprevir and changes in the glomerular filtration rate have on ribavirin plasma levels. The minimum concentrations of ribavirin in plasma (ribavirin Cmin) determined during triple therapy including telaprevir were compared with those observed after telaprevir withdrawal and those observed in the same subjects and in a large cohort during a previous course of pegIFN-α plus ribavirin. Intensive pharmacokinetic sampling for ribavirin was performed at steady state during the triple-therapy phase. Ribavirin levels were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Twenty-seven HCV-1/HIV-coinfected patients were enrolled. The median ribavirin Cmin for triple therapy (4.08 μg/ml; range, 2.14 to 5.56 μg/ml) was higher than that observed after telaprevir withdrawal (1.96 μg/ml; range, 0.41 to 3.45 μg/ml) (P < 0.001) and that observed for 125 HCV-1/HIV-coinfected patients treated only with pegIFN-α plus ribavirin (1.65 μg/ml; range, 0.41 to 3.56 μg/ml) (P < 0.001). The estimated glomerular filtration rate (eGFR) decreased >20% from the baseline value in 11 of 27 patients and became normal after telaprevir removal in almost in all cases. There was a negative correlation between eGFR and ribavirin clearance (r² = 0.257; P = 0.064) but not the ribavirin area under the concentration-time curve from 0 to 12 h (AUC₀–12) (r² = 0.001; P = 0.455). Thus, there is a significant pharmacokinetic interaction between telaprevir and ribavirin that results in very high ribavirin levels, which explains the excess of toxicity observed with this drug combination. A blockade of the proximal tubular transporters might be implicated in both the increase in plasma creatinine and the high ribavirin levels. (This study has been registered at ClinicalTrials.gov under registration no. NCT01818856.)

There is an extensive amount of literature on the pharmacokinetics and pharmacodynamics of ribavirin (Rbv) showing discordant data about the relationship between Rbv plasma concentrations and efficacy during dual therapy (DT) with pegylated alpha interferon (pegIFN-α) plus Rbv. However, all studies agree about the association between Rbv plasma levels and the occurrence and severity of anemia (1–13). In the recent past, the NS3/4A protease inhibitors boceprevir and telaprevir (TVR) were incorporated into the armamentarium for the treatment of chronic hepatitis C virus genotype 1 (HCV-1) infection. The recommended Rbv dosing during triple therapy (TT) was the same as in DT, since pharmacological interactions between pegIFN-α, Rbv, and TVR were not expected (14, 15). However, a higher incidence and severity of anemia were observed in both mono- and HCV/HIV-coinfected patients during the course of TT. Initially, the higher anemia rates were ascribed to an additive effect of Rbv and the NS3/4A protease inhibitors on erythropoiesis (16, 17). Later, some studies suggested that the decrease in the estimated glomerular filtration rate (eGFR) observed in some patients during TT was the cause of the accumulation of Rbv and the higher incidence of anemia (18–22); K. P. Hammond, L. Jimmerson, C. E. MacBrayne, M. Ray, L. Bushman, J. R. Burton, F. Baouchi-Mokrane, G. T. Everson, P. L. Anderson, and J. J. Kiser, presented at the 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, GA, 2013). The aim of this study was to assess the impacts that concomitant administration of telaprevir and changes in the glomerular filtration rate have on ribavirin plasma levels.

MATERIALS AND METHODS

Study population. The patients included in this study were adult Cauca- sian HCV-1/HIV-coinfected subjects who started a planned TT-TVr regi- men with standard dosing of pegIFN-α 2a (180 μg weekly), weight-based Rbv dosing (800 to 1,200 mg/day), and TVR (1,125 mg/12 h) for 12 weeks, followed by 36 additional weeks of pegIFN-α 2a plus Rbv, from October 2012 to October 2013. They were treated according to the criteria of the responsible physicians under routine clinical care conditions. Both TVR and Rbv were recommended to be taken after breakfast and dinner. Liver stiffness was determined by hepatic transient elastography (FibroScan; Echosens, Paris, France), with values of ≥14.6 kPa indicating cirrhosis (23).

Clinical assessments and blood sampling were performed at baseline, every 4 weeks until week 12, and every 3 months thereafter. For determination of the minimum concentration of Rbv in plasma (Cmin), blood samples were drawn at each of these visits, 11.45 to 12.15 h after the previous Rbv dose (otherwise, the samples were discarded). Dropout data were included up to the last available visit for individuals on treatment. The Rbv levels before week 4 were excluded from the analysis, as we had previously observed that Rbv concentrations were significantly lower dur-
ing the first 2 weeks of treatment and became stable from week 4 onward (13).

Additionally, intensive pharmacokinetic profiles collected over 12 h from 14 patients receiving at least 4 weeks of Rbv concomitantly with TVR were analyzed. Blood samples were obtained immediately before and 1, 2, 3, 4, 5, 6, 8, 10, and 12 h after supervised drug intake following a standard breakfast (ClinicalTrials.gov registration no. NCT01818856) (24). This study was approved by the Spanish Agency for Medicine and Healthcare Products and by a central ethics committee (Comité Autonómico de Ensayos Clínicos, Consejería de Salud, Junta de Andalucía). The study adhered to the Declaration of Helsinki and the current guidelines on good clinical practice. All the patients provided informed consent.

**Laboratory tests.** The eGFR was measured using the Cockroft–Gault formula, adjusted by body surface area, and expressed in milliliters per minute per 1.73 m². Hepatitis C virus genotypes and subtypes were determined using a reverse hybridization assay (Inno-Lipa HCV II; Bayer, Barcelona, Spain). The plasma HCV RNA levels were measured using a quantitative PCR assay (detection limit of 15 IU/ml) (CobasTaqMan; Roche Diagnostics, Basel, Switzerland), respectively. The interleukin-28B (IL-28B) single-nucleotide polymorphism (SNP) rs12980274 (previous intravenous drug use/other) was genotyped using a TaqMan 5’-allele discrimination assay (Applied Biosystems, Foster City, CA) on DNAs isolated from whole-blood samples or peripheral blood mononuclear cells (PBMCs), where available, as previously described (25).

Plasma concentrations of Rbv were measured in samples stored at −80°C by a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay with a lower limit of quantification of 90 ng/ml. The accuracy and precision were 100% ± 10% and <10%, respectively. Standard curves were highly linear over the range of 0.15 to 20 μg/ml. The recovery of Rbv from human plasma was 96.8% ± 3.75%. The mean intra- and interassay coefficients of variation were 7.20 and 8.20%, respectively. Validation of the method was performed by FDA guidelines, and the results met the acceptance criteria.

**Statistical analysis.** Continuous variables were expressed as medians or geometric means (GM) (ranges), and categorical variables were expressed as numbers of cases and percentages. Continuous variables were compared using the Mann–Whitney U test or the Wilcoxon signed-rank test, as appropriate, and categorical variables were compared by applying the χ² test. The correlations between different quantitative variables were assessed by using Spearman’s rank correlation coefficients as appropriate. Linear regression analyses were used to assess the relationships between different quantitative variables. During the triple-therapy course, Rbv plasma concentrations were compared between the period when Rbv was given concomitantly with TVR (weeks 0 to 12) and the period after TVR discontinuation (week 13 forward), using analyses of repeated measures. Additionally, the Rbv levels during TT were compared with both those observed in the same subjects during a previous DT course and those for a cohort of 125 adult Caucasian HCV-1/HIV-coinfected patients treated with pegIFN-α plus Rbv, without Rbv dose reduction during the DT course, which were measured by reverse-phase high-performance liquid chromatography in the same hospital (13).

**RESULTS**

A total of 113 Rbv Cₘᵦᵢₜ determinations were performed on samples taken from 27 HCV/HIV-coinfected subjects throughout the TT-TVR courses, with a median of 3 (1 to 4) samples per patient from weeks 4 to 12 and a median of 1 (0 to 3) sample per patient from week 18 onward (after the TVR withdrawal). The main subject characteristics and Rbv doses are shown in Table 1. Both the
weight and the body mass index (BMI) were significantly lower in patients previously treated with DT. This fact, if anything, would favor smaller differences in Rbv levels between the DT and TT cohorts, which is quite the opposite from what we found.

**Ribavirin C_{min} levels in the entire cohort.** Overall, the median Rbv C_{min} for patients on triple therapy (4.08 μg/ml; range, 2.14 to 5.56 μg/ml) was higher than that for patients following TVR withdrawal (1.96 μg/ml; range, 0.41 to 3.45 μg/ml) (P < 0.001) and that for the 125 patients in the previous cohort treated with pegIFN-α plus Rbv (1.65 μg/ml; range, 0.41 to 5.56 μg/ml) (P < 0.001) (Fig. 1). During the coadministration of Rbv and TVR, the Rbv C_{min} was higher in subjects taking concomitant tenofovir treatment (4.64 μg/ml; range, 2.50 to 5.71 μg/ml) than in those receiving abacavir or antiretroviral treatments without analogs (3.81 μg/ml; range, 2.14 to 5.39 μg/ml) (P = 0.085), although the difference did not reach statistical significance.

There was a close correlation between the Rbv C_{min} at week 4 and the GM of Rbv levels throughout weeks 4 to 12 (r = 0.885; P < 0.001). However, no relationships were found between weight, BMI, or Rbv dosing (milligrams per kilogram of body weight per day) and the Rbv C_{min} levels. Similar intrapatient variabilities of Rbv levels were found in the previous DT cohort (27.9%; interquartile range [IQR], 16.7 to 40.2%; range, 2 to 91%) and in the period of 12 weeks on TT-TVR (18.2%; IQR, 13.8 to 32.4%; range, 7 to 66%) (P = 0.935). Likewise, the interindividual variabilities for the previous cohort and the present one were 46.7% and 33.7%, respectively.

**Ribavirin C_{min} levels during and after telaprevir coadministration.** Eleven subjects stopped treatment between weeks 4 and 22 due to adverse effects or a lack of efficacy, and no Rbv determinations were available for them following TVR withdrawal. Four patients completed the full treatment but had their Rbv dose reduced by a median of 200 mg/day (200 to 400 mg/day) from week 13 onward, due to severe anemia. Thus, Rbv levels were available for 12 patients throughout the complete 48 weeks of treatment, before and following TVR withdrawal, without changes in the Rbv dosing. For these patients, the median C_{min} was 3.87 μg/ml (2.14 to 7.1 μg/ml) during the coadministration of Rbv and TVR and 1.96 μg/ml (0.41 to 3.45 μg/ml) after TVR withdrawal (P = 0.005). For 6 of these 12 subjects, the Rbv dose was also unchanged during the previous DT. The Rbv C_{min} values during the Rbv and TVR coadministration were significantly higher than those during the earlier DT regimen and after TVR withdrawal, despite a lower median level of Rbv per kilogram per day during TT-TVR than during DT (14.9 mg/kg/day [12.5 to 20.3 mg/kg/day] versus 12.65 mg/kg/day [11.2 to 15.6 mg/kg/day]) (P = 0.043) (Fig. 2).

**Pharmacokinetic profile of ribavirin during telaprevir coadministration.** The estimated Rbv pharmacokinetic parameters and curve profiles for 14 patients are shown in Fig. 3. There were poor correlations between the Rbv AUC_{0-12} and C_{max} (r^2 = 0.396; P = 0.008) or C_{min} (r^2 = 0.223; P = 0.044), as well as between C_{max} and C_{min} (r^2 = 0.285; P = 0.025). Both C_{max} and C_{min} were negatively correlated with the Rbv CL values (r^2 = 0.555 and 0.555, respectively; P = 0.001). Moreover, there were weak negative correlations between eGFR and Rbv CL (r^2 = 0.257; P = 0.064) and between eGFR and C_{max} (r^2 = 0.249; P = 0.035) but not between eGFR and AUC_{0-12} (r^2 = 0.001; P = 0.455) or C_{min} (r^2 = 0.005; P = 0.455).

**Renal function during and after telaprevir administration.** In the previous cohort treated with DT, there were no significant
changes in plasma creatinine or eGFR during therapy with pegIFN-α plus Rbv. Nevertheless, during the TT-TVR regimen, the eGFR decreased from weeks 0 to 12, returning to baseline values following TVR withdrawal (Table 2). The eGFR decreased >20% from the baseline value for 11 of the 27 patients (40.7%), but this was reversible after week 12 in almost all cases. Negative correlations were also found between the Rbv C_{\text{min}} at week 4 or the GM of the determinations from weeks 4 to 12 and the eGFR at these points (r^2 = 0.313 and P = 0.019 for both correlations) during the TT-TVR regimen.

**DISCUSSION**

The addition of a protease inhibitor (boceprevir or TVR) to the classic therapy with pegIFN-α plus Rbv was an important event in the treatment of HCV-1. However, higher incidences and severities of hematologic toxicity were unexpectedly observed with these regimens. It has been suggested that the decrease in the eGFR occurring in some patients on TT-TVR might be the reason for Rbv accumulation and the higher incidence of anemia (19–21). We provide evidence that plasma Rbv levels increased significantly when DT was combined with TVR compared to (i) the Rbv levels observed in the same patients after TVR withdrawal (week 13 onward), (ii) the levels of Rbv observed during previous DT in the same subjects, and (iii) the Rbv concentrations observed in a large cohort of patients treated with DT in the same hospital.

There are several possibilities regarding the underlying mechanism of interaction between Rbv and TVR. First, TVR might cause an increase in the bioavailability of Rbv, which is transported actively by gastrointestinal N1 sodium-dependent nucleoside transporters in the proximal small bowel (26, 27). Several physiopathologic conditions and drugs can increase the activity of nucleoside transporters, but it is improbable that TVR has a potential inducer effect on these transporters.

Second, TVR might induce a decrease in the renal excretion of Rbv. Rbv has two pathways of cellular metabolism: a reversible phosphorylation pathway and a degradative pathway to yield a triazole carboxylic acid metabolite, with both Rbv and its main metabolite being renally excreted (26). A decline in renal function along with the weight loss frequently observed during pegIFN-α therapy would give rise to the accumulation of Rbv. However, based on the weak negative correlation between eGFR and Rbv CL and the null correlation between eGFR and Rbv exposure, we believe that the moderate decrease in the eGFR observed during the current TT-TVR regimen does not fully explain the high Rbv levels observed (28).

It was recently reported that TVR significantly inhibits the organic cation transporter 2 (OCT2) and multidrug and toxin extrusion protein 1 (MATE1) human renal drug transporters (29). We believe that the inhibition of these transporters in renal tubular cells by TVR might explain, at least in part, both the increase in plasma creatinine and the high Rbv level, which also occur with other drugs, such as cimetidine, cobicistat, dolutegravir, and ritonavir (30, 31). This hypothesis is supported by the weak rela-

### TABLE 2 Evolution of plasma creatinine level and eGFR during telaprevir-based triple therapy (weeks 0 to 12) and the pegIFN-α-plus-ribavirin continuation phase (weeks 13 to 48) in the current cohort and during pegIFN-α-plus-ribavirin treatment in the previous cohort

<table>
<thead>
<tr>
<th>Week (no. of patients/no. of determinations)</th>
<th>Current cohort (pegIFN-α + Rbv + TVR)</th>
<th>Previous cohort (pegIFN-α + Rbv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR) (range) creatinine level (µg/ml)</td>
<td>Median (IQR) (range) eGFR (ml/min/1.73 m²)</td>
<td>Median (IQR) (range) eGFR (ml/min/1.73 m²)</td>
</tr>
<tr>
<td>P value*</td>
<td>P value*</td>
<td>P value*</td>
</tr>
<tr>
<td>0 (27/131)</td>
<td>0.88 (0.81–0.98)</td>
<td>99.3 (85.4–99.3)</td>
</tr>
<tr>
<td></td>
<td>(0.61–1.26)</td>
<td>(75.3–149.6)</td>
</tr>
<tr>
<td>4 (27/126)</td>
<td>0.98 (0.86–1.09)</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>(0.50–1.44)</td>
<td>94.9 (77.1–109.0)</td>
</tr>
<tr>
<td>8 (26/103)</td>
<td>1.06 (0.85–1.16)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(0.67–2.45)</td>
<td>85.6 (72.0–85.8)</td>
</tr>
<tr>
<td>12 (23/104)</td>
<td>0.99 (0.81–1.16)</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>(0.67–2.55)</td>
<td>93.5 (76–115.3)</td>
</tr>
<tr>
<td>24 (16/79)</td>
<td>0.85 (0.75–0.99)</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>(0.61–1.63)</td>
<td>97.8 (84.2–132.5)</td>
</tr>
<tr>
<td>48 (14/63)</td>
<td>0.98 (0.84–1.21)</td>
<td>0.649</td>
</tr>
<tr>
<td></td>
<td>(0.65–1.33)</td>
<td>90.1 (72.5–114.1)</td>
</tr>
</tbody>
</table>

*P values are for comparisons with baseline values.
tionship between Rbv CL and eGFR and by the normalization of Rbv levels and the reversal of the eGFR impairment after week 12, which coincides with TVR withdrawal.

Thus, the impairment of the eGFR observed in some subjects during the TT-TVTR regimen might be due to an altered proximal tubular secretion of creatinine through the inhibition of drug transporters by TVR rather than due to true renal dysfunction. To our knowledge, it is unknown whether Rbv is eliminated only by glomerular filtration or by tubular secretion as well. However, this is a plausible hypothesis, as other nucleoside analogues undergo tubule-mediated elimination by different organic anion and cation transporters and multidrug and toxin extrusion proteins (32–35).

On the other hand, the full Rbv pharmacokinetic profiles showed that Cmax, Cmin, and AUC0–12 were much higher and the Rbv CL lower during TVR coadministration than those reported in the literature (26, 36–38). The poor correlation observed between the Cmin and the Rbv exposure over the dosing interval (AUC0–12) is worth noting, making Cmin a poor parameter for Rbv therapeutic monitoring, an issue that has not been clarified in previous pharmacokinetic studies of Rbv.

A limitation of our study might be the use of historical data for some comparisons, but it does not undermine the main findings. Currently, TVR has been replaced almost completely by second-generation hepatitis C virus NS5A/4A protease inhibitors, which have a lower incidence of anemia, but this type of interaction should be explored for any regimen including Rbv.

In summary, there is a significant pharmacokinetic interaction between TVR and Rbv in HCV/HIV-coinfected patients that results in very high Rbv levels, which explains the excess hematological toxicity observed with this drug combination. A blockade of the proximal tubular OCT2 and MATE1 transporters might be the underlying mechanism for both the increase in plasma creatinine and the high Rbv levels.

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The other authors have no conflicts to declare.

REFERENCES


