VIEW POINT

Optimizing ribavirin dosage: A new challenge to improve treatment efficacy in genotype 1 hepatitis C patients

Optimiser les doses de ribavirine : un nouveau challenge pour augmenter l’efficacité du traitement chez les patients infectés par le virus de l’hépatite C de gênotype 1

V. Loustaud-Ratti\textsuperscript{a,∗}, F. Stanke-Labesque\textsuperscript{b}, P. Marquet\textsuperscript{c}, M.-C. Gagnieu\textsuperscript{d}, M. Maynard\textsuperscript{e}, G. Babany\textsuperscript{f}, C. Trépo\textsuperscript{e}, The French Ribavirin Group OPTIRIB\textsuperscript{1}

\textsuperscript{a} Service de médecine interne et fédération des hépatites, CHU Dupuytren, 2, avenue Martin-Luther-King, 87042 Limoges cedex, France
\textsuperscript{b} Inserm ERI17, service de pharmacologie-toxicologie, CHU de Grenoble, université de Grenoble, Grenoble, France
\textsuperscript{c} Inserm U850, service de pharmacologie et toxicologie, pharmacovigilance, CHU de Limoges, université de Limoges, Limoges, France
\textsuperscript{d} Service de pharmacologie et toxicologie, pharmacovigilance, Hôtel-Dieu, Lyon, France
\textsuperscript{e} Service d’hépatogastro-entérologie, Hôtel-Dieu, Lyon, France
\textsuperscript{f} Roche, Neuilly-sur-Seine, France

Available online 28 May 2009

Introduction

The standard antiviral treatment for chronic hepatitis C virus (HCV) infection combines pegylated interferon-alfa and ribavirin. The synergy between ribavirin and peginterferon can double the sustained virological response (SVR) rate obtained with peginterferon on its own. Ribavirin acts by sustaining the virological response to peginterferon-alfa [1]. However, although the drug combination is highly effective in patients infected with genotype 2 or 3 HCV, nearly half of the genotype 1 HCV patients fail to achieve a SVR [2].

The total daily dose of ribavirin (>10.6 mg/kg of body weight), especially during the first 3 months of treatment, is predictive of the SVR and ribavirin has to be administered throughout the whole course of treatment [1]. In addition, Lindahl et al. [3] have suggested that the early use of very high-dose ribavirin (mean daily dose of 2.5 g [range: 1.6—3.6 g]) led to higher SVR rates in genotype 1 patients. This suggests that optimizing a given patient’s...
Ribavirin dose by monitoring drug blood levels could be the key strategy for improving SVR rates, especially in difficult-to-treat patients. This underscores the need for a better understanding of ribavirin pharmacokinetics and its pharmacokinetic–pharmacodynamic (PK–PD) relationships.

**Ribavirin dose-adjustment techniques**

Individual dose adjustment could be based on individual factors linked to the dose–concentration relationship. Bruchfeld et al. recommended that ribavirin dosage be mainly adjusted according to creatinine clearance and not, as done in practice, to body weight. However, in their study, the effect of renal function on ribavirin clearance was only apparent when creatinine clearance was less than 34 mL/min [4], which is only seen in a minority of patients.

Glue et al. [5] found that hepatic dysfunction had no substantial influence on the apparent clearance of ribavirin and thus, was not a useful candidate. Population pharmacokinetic studies investigating the potential covariates that influence ribavirin pharmacokinetics have demonstrated that body weight, gender, age and creatinine clearance all affect ribavirin concentrations, but these four variables explained only 27% of the interindividual variability seen in ribavirin clearance [6].

For this reason, therapeutic drug monitoring of ribavirin – based on drug exposure measurements – is considered a feasible option for improving treatment efficacy. Indeed, ribavirin exhibits a profile typical for therapeutic drug monitoring as pharmacokinetic studies have demonstrated that ribavirin bioavailability displays wide interindividual variability and that its plasma concentrations correlate poorly with the daily ribavirin dose, even after adjustment for body weight [7—9]. In addition, a concentration–effect relationship between ribavirin exposure and SVR [7,8,10] has also been suggested.

**Tools for measuring ribavirin exposure**

Following a single oral dose, ribavirin plasma concentration exhibits a three-phase profile [11]:

- a rapid-absorption phase with a mean time to maximum concentration (t_{max}) of about 1.5 h;
- a rapid-distribution phase (half-life of approximately 3.7 h);
- a long terminal elimination phase, the last measurable concentration time-point being at around 100 h postdose.

Following multiple dosing, ribavirin gradually accumulates in plasma to reach an asymptotic steady-state concentration after around 4 weeks.

Initial attempts to monitor ribavirin blood levels were based on single-point or sparse sampling at weeks 4, 12 or 24 after starting treatment. A retrospective pilot study reported that at weeks 12 and 24, ribavirin concentrations (measured 2—4 h after the morning dose, close to its peak concentration) were significantly higher in patients with a SVR than in those without [8]. Jen et al. [7] demonstrated that serum ribavirin concentrations at week 4 were linked to SVR, but suggested that it was less influential than the HCV genotype or viral load. However, the interval between the last dose of ribavirin and blood sampling was not predetermined in their protocol. Recently, another study found a relationship between trough concentrations measured at week 4 and SVR in naïve, monoinfected genotype 1 HCV patients [12]. Finally, a pilot study involving patients with genotype 1 HCV and high viral loads demonstrated that achieving high concentrations of ribavirin (>15 μM) before week 12, using high doses of ribavirin (mean daily dose: 2.5 g [range: 1.6—3.6 g]), led to SVRs in nine of 10 patients [3] – albeit with frequent and serious side effects.

However, patients’ global exposure to ribavirin, as evaluated by the area under the curve (AUC), appears to be more relevant to the exposure–effect relationship than any single timepoint. Nevertheless, the number of blood samples and sampling times required to adequately estimate global ribavirin exposure and how early to carry out such an evaluation remain subjects of debate. Indeed, studies based on ribavirin AUCs have had conflicting results. Recently, it was found that, in patients infected with genotype 1 HCV, ribavirin plasma exposure after the first dose was significantly and strongly linked to SVR [10]. In that study, conducted in a real-life situation in which patients were given ribavirin twice a day, both the interdose (AUC_{0—12h}) and abbreviated (AUC_{0—4h}) AUCs were used as indices of drug exposure. After the first dose, the AUC_{0—12h} was only slightly affected by the elimination phase (due to the long half-life of the drug), while the abbreviated AUC_{0—4h} was clearly related to ribavirin absorption and distribution. In contrast, in the Dahari et al.’s study [13] of HCV/human immunodeficiency virus (HIV)-infected patients, ribavirin AUCs measured after repeated dosing were significantly lower in the sustained virological responders than in nonresponders. In that study, AUCs were measured during the first 8 days of treatment and, so, reflected not only the absorption and distribution phases, but most of the elimination phase, too. In the present study, the concentration variability seen in the absorption and distribution phases was less evident on the concentration–time curves obtained at steady-state (weeks 12 and 24), which might explain why the AUCs measured at week 12 were not significantly linked to SVR rates, consistent with previous findings that trough concentrations at either week 4 or 12 are not strong predictors of SVR [10]. This was also in accordance with the results of in vitro studies showing that intracellular concentrations of ribavirin were at maximum at 6 h postdose [11]. Finally, based on receiver operating characteristic (ROC) curve analyses, a minimum AUC_{0—4h} threshold of 1755 μg/h/L at day 0 was proposed as the target for ribavirin dose adjustment [10].

The relationship between ribavirin concentrations and an early virological response is unclear. In one retrospective study, Donnerer et al. found no correlation between ribavirin levels at week 12 and an early virological response [14]. In contrast, recent findings in HCV/HIV coinfected patients suggest that high ribavirin concentrations could improve early virological response rates [15]. Ribavirin plasma AUC_{0—12h} and AUC_{0—4h} after the first dose were both significantly linked to early virological responses, but to a lesser extent compared with SVR and rapid virological responses [10]. These discrepancies could be explained by the low positive predictive value of the early virological response for SVR, the retrospective design of most of these
studies and/or the lack of information on the times of sample collection in some.

In general, the above-mentioned studies found a relatively strong link (depending on the strategy employed) between ribavirin exposure indices and SVR, which is ultimately the principal objective of treatment in HCV infection. Very-early ribavirin pharmacokinetics may be a strong determinant of treatment outcome, and an early predictor of SVR would ideally be related to early adjustment of the ribavirin dose.

In which patients should ribavirin exposure be measured?

A recent study of 35 HCV/HIV coinfected patients [16] suggested that the influence of ribavirin concentrations on the SVR could be linked to the HCV genotype (a clear influence on viral response was only seen for genotypes 1 and 4). However, the Scandinavian NORDynamic trial, involving 382 genotype 2 or 3 HCV monoinfected patients, revealed a statistical relationship between residual ribavirin concentrations at week 4 of treatment and SVR. The positive predictive value for SVR at week 4 was 91% for a ribavirin concentration greater than 2 mg/L (P = 0.02) [17]. This suggested that ribavirin residual concentrations could have an impact on SVR in all genotypes, but that such an impact was probably greater in genotype 1 patients. However, no ribavirin AUC data are available for genotype 2 or 3 patients.

The link between ribavirin and hematological toxicity

An association between ribavirin concentration and toxicity in the first 3 months of treatment is well established, at least for anemia [3]. However, the negative correlation between a single concentration at week 4 and the week 4 hemoglobin nadir is highly variable [18]. It was also found that ribavirin AUC values at day 0 (but not at week 12) correlated with hemoglobin levels at week 12 [10]. Arase et al. have also demonstrated that high ribavirin trough concentrations at week 8 (>3.5 mg/L) were linked to higher rates of ribavirin discontinuation because of anemia and suggested that this limit not be exceeded for safety reasons [19]. In fact, the relationship between ribavirin concentration and side effects is another good reason for therapeutic drug monitoring.

Early (day 0) ribavirin drug monitoring

Ribavirin is a nucleoside analog with an antiviral mechanism that is still controversial in terms of its direct inhibition of HCV replication, inosine monophosphate dehydrogenase inhibition leading to GTP depletion, modulation of T helper-1/helper-2 cell balance and positive effects on apoptotic pathways [20]. Also, ribavirin might have mutagenic effects, inhibiting the ability of progeny subgenomic replicons to transfect new cells [21].

A recent pathophysiological paper by Feld et al. [22] supports the use of very-early ribavirin dose adjustment, as it found that ribavirin could enhance both the induction of genes by peginterferon and the response of these interferon-stimulated genes to peginterferon; thereby, making cells more responsive to interferon and increasing its endogenous production.

Ribavirin concentration measurements

Several analytical methods have been developed to determine ribavirin levels in biological fluids [23], including radioimmunoassay, capillary electrophoresis or HPLC coupled with mass spectrometry or UV detection. The choice of plasma or serum for ribavirin concentration measurements may also be important; ribavirin levels were more reproducible in plasma than in serum in pharmacokinetic studies using intensive sampling [7]. This apparent discrepancy might be due to the poorer stability of serum levels during the blood-clotting phase at ambient temperatures. Indeed, as ribavirin can be transferred between blood cells and serum or plasma in the sampling tube, samples should be put on ice immediately in the laboratory (the maximum time is 0.5 h) and centrifuged. After that, the plasma or serum samples can be stored at −20 °C (Table 1).

However, determining intraerythrocyte ribavirin concentrations might be a more relevant approach than measuring plasma levels, as intraerythrocyte ribavirin accumulation early on during treatment would be higher in patients likely to achieve SVR [13]. However, the irreversible intraerythrocyte accumulation of ribavirin–triphosphate is probably not representative of the drug turnover in other target cells such as hepatocytes, where ribavirin activation into triphosphate metabolites is reversible. Nevertheless, intraerythrocyte determination of ribavirin needs to be further investigated, as there is no evidence so far that it is a better approach than plasma concentrations.

<table>
<thead>
<tr>
<th>Table 1: Practical aspects of ribavirin plasma-concentration analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing</strong></td>
</tr>
<tr>
<td><strong>Medium</strong></td>
</tr>
<tr>
<td><strong>Storage</strong></td>
</tr>
<tr>
<td><strong>Dosage</strong></td>
</tr>
<tr>
<td><strong>Location</strong></td>
</tr>
<tr>
<td><strong>Results</strong></td>
</tr>
<tr>
<td><strong>HPLC:</strong></td>
</tr>
</tbody>
</table>
In summary, ribavirin concentrations can be measured in either plasma or serum and by HPLC coupled with either UV or MS detection, but the key point is to reduce the time between blood sampling and centrifugation to less than 0.5 h.

**Conclusion**

The aim of ribavirin therapeutic monitoring is to allow individual adjustments of the dose to offer each patient the greatest chances of achieving SVR with acceptable side effects. This means that early and robust pharmacokinetic predictors of SVR should be used. Both the AUC$_{0-12h}$ and AUC$_{0-4h}$ after the first ribavirin dose are significantly and strongly related to SVR [10]. AUC$_{0-4h}$ can be estimated using three blood samples (taken at 0.5, 1, and 2 h after the first dose) and Bayesian estimation and a predetermined threshold have also been proposed. An individually adapted ribavirin dose to reach this threshold can be proposed before day 7 of treatment, an approach that is soon to be validated prospectively in the French randomized controlled trial Ribajuste, designed to evaluate the efficiency and tolerability of very-early individual monitoring of ribavirin in naive genotype 1 HCV patients. Ribavirin monitoring may also be useful in future tritherapies using specifically targeted antivirals, as ribavirin is essential for preventing relapses with different therapeutic combinations and, in particular, antiprotease inhibitors [24].

The use of ribavirin trough concentrations at week 4 or 12 to predict SVR appears to be less valid [9], although trough concentrations at weeks 4 and 8 could help to prevent the potential toxicity of ribavirin in certain individuals when administered at high doses to achieve the target AUC and to confirm patient compliance.

**References**


