Usefulness of monitoring ribavirin plasma concentrations to improve treatment response in patients with chronic hepatitis C

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Ribavirin in combination with pegylated interferon α is the current standard treatment for chronic hepatitis C. Adequate exposure to ribavirin seems crucial for achieving the best virological response. However, anaemia is a frequent, dose-dependent limiting side effect of ribavirin use. Therefore, therapeutic drug monitoring of ribavirin plasma concentrations could be a useful tool for individualizing ribavirin dosing. Herein, we review the relationship between ribavirin plasma concentrations and both virological response and toxicity, in order to define an optimal therapeutic range for ribavirin.

Keywords: HCV, HIV, therapeutic drug monitoring, pharmacokinetics

Introduction

Chronic hepatitis C virus (HCV) infection affects approximately 200 million people worldwide and is one of the leading causes of chronic liver disease and indication for liver transplantation.1 Ribavirin in combination with pegylated interferon α is currently the standard treatment for chronic hepatitis C. Sustained virological response (SVR) is achieved in 50% to 60% of HCV mono-infected patients.1–4 The recommended ribavirin dose is based on body weight, being generally 1000 mg/day if ≤ 75 kg and 1200 mg/day if > 75 kg. In subjects with very low or very high body weights, lower or higher ribavirin doses may be considered. Treatment is currently based on HCV genotype and rapid virological response (RVR), ranging from 3 to 18 months.5,6 Patients infected with HCV genotype 2 or 3 and RVR could benefit from shorter courses of therapy (3–4 months).7,8 In contrast, subjects infected with HCV genotype 1 or 4 lacking RVR may benefit from a longer course of therapy (12–18 months).9,10 Similar observations are being reproduced in HIV/HCV co-infected patients, although response rates tend to be lower in this population.11

Monitoring drug concentrations of some antiretrovirals has been beneficial in the clinical management of HIV patients, permitting the improvement of the chances of viral suppression while minimizing the risk of toxicities.12–14 However, the usefulness of monitoring nucleoside analogue plasma levels is unclear since they need intracellular activation, and correlation between plasma drug levels and either activity or toxicity is indirect. In chronic hepatitis C, the mechanism of action of ribavirin, a guanosine analogue, is still unknown, and so far it is unclear to what extent phosphorylated metabolites may mediate ribavirin antiviral effects and/or toxicities. Herein, we summarize the most relevant studies that have assessed the usefulness of therapeutic drug monitoring (TDM) for ribavirin.

Mechanism of action of HCV medications

Interferon α has a direct antiviral action by producing proteins derived from induction of interferon-stimulated genes, which blocks viral replication. Furthermore, it shows an immunomodulatory effect on adaptive and innate immune responses.15,16 Interferon therapy results in a biphasic viral kinetic decay with a very rapid reduction in HCV viraemia during the first 24–36 h after administration of the first dose and again within 4–16 weeks of treatment.

Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is a synthetic guanosine analogue that exerts antiviral activity against HCV. How ribavirin augments the response rate to interferon is not well known, but multiple mechanisms have been proposed, including direct inhibition of HCV replication, inosine-monophosphate-dehydrogenase inhibition, hypermutation and immunomodulation.15 The effect of ribavirin depends on the effectiveness of interferon in blocking viral production. When interferon effectiveness is high, such as in high-dose induction protocols or in the treatment of HCV genotype 2 or 3, ribavirin is predicted to have a minimal effect, since virion production is efficiently blocked. In contrast, when the effectiveness of interferon is low, ribavirin is predicted to substantially decrease viral infectivity and to enhance the second slope of viral decay.16,17 In this regard, ribavirin accumulation and
steady-state concentrations will play an important role in the achievement of SVR.

Adverse effects of HCV medications

Combination antiviral therapy for chronic hepatitis C is associated with significant side effects such as anaemia, neutropenia, thrombocytopenia, skin rashes, anorexia and depression. Specially, anaemia may require ribavirin dose reductions or even discontinuation. Both ribavirin and, to a less extent, pegylated interferon α may reduce haemoglobin (Hb) concentration and result in anaemia by different mechanisms. Ribavirin causes haemolytic anaemia by the accumulation of ribavirin-triphosphate and posterior depletion of intracellular ATP in red blood cells, while interferon leads to bone marrow suppression. This additive adverse effect is responsible for an average decline in Hb levels of 2–3 g/dL during the first 12 weeks of HCV therapy. In ~10% of the patients, Hb drops to <10 g/dL, requiring ribavirin dose reductions with the potential risk of an impaired SVR. Predictors of a higher risk for developing anaemia include older age, female gender, low platelet counts and low body weight.

Response to hepatitis C therapy

Baseline serum HCV-RNA and HCV genotype are the main predictors of SVR to pegylated interferon α plus ribavirin in patients with chronic hepatitis C. Several other variables, however, have been similarly associated with a better treatment outcome. They can be grouped into three categories, as belonging to the host (younger age, non-black ethnicity, lower body mass index and lack of insulin resistance), the liver (elevated aminotransferase (ALT), lack or minimal hepatic fibrosis) and the treatment modality (use of optimal doses of pegylated interferon α and/or ribavirin, enough length of therapy and good adherence).

In HIV/HCV co-infected patients, the risk of drug interactions between antiretroviral drugs and ribavirin might increase the toxicity and compromise the virological response. Ribavirin is also a nucleoside analogue, it has been suggested that concomitant use of ribavirin and nucleoside reverse transcriptase inhibitors (NRTIs) may result in an adverse additive effect on mitochondrial toxicity. Thus, concomitant use of didanosine and ribavirin is not recommended because there have been reports of fatal hepatic failure, peripheral neuropathy, pancreatitis and lactic acidosis. Ribavirin should be used with caution when co-administered with other NRTIs (e.g. stavudine). Concomitant use of ribavirin and zidovudine should be avoided, or used with caution and increased monitoring due to an increased incidence of severe anaemia.

In contrast, antiretroviral drugs may affect the response to HCV through inhibitory competition phenomena between some nucleoside analogues and ribavirin. This could be the case of the association between abacavir use and lower SVR rate that has been observed mainly among patients treated with lower doses of ribavirin and lower ribavirin concentrations. As both drugs are guanosine analogues and share intracellular phosphorylation pathways, a potential competition for the same enzymes may interfere with their respective antiviral activity.

Hepatitis C treatment monitoring

The kinetics of HCV-RNA in response to pegylated interferon α plus ribavirin is a reliable indicator of treatment efficacy. The best positive predictive value for SVR is achieved when a negative serum HCV-RNA is attained at week 4 (RVR), while the best negative predictive value for SVR is seen when HCV-RNA falls by <2 log IU/mL at week 12 (early virological response, EVR). As will be discussed later, TDM of ribavirin could be a useful tool to tailor ribavirin dosages in single patients in order to enhance the chances of SVR with the lowest risk of toxicity. The establishment of a therapeutic window for ribavirin levels, defined as the range of concentrations associated with the optimal efficacy/toxicity ratio, may be of great value for the appropriate use of the drug.

Ribavirin pharmacokinetics

Ribavirin shows rapid absorption and distribution phases and a long terminal clearance phase. Its oral bioavailability is low (45% to 65%) because of first-pass metabolism and is distributed minimally bound to plasma proteins. The volume of distribution is high (several thousand litres). Transport to non-plasma compartments has been mainly studied in red cells, and the ratio of distribution among whole blood and plasma is 60:1. Ribavirin appears to distribute slowly into the CSF, concentrations being up to 70% of plasma concentrations. At this moment, it is unclear whether ribavirin crosses the placenta or distributes into milk in humans. With respect to elimination, ribavirin has two pathways of metabolism: a reversible phosphorylation pathway to mono-, di- and triphosphates (active) and a degradative pathway involving deribosylation and amide hydrolysis to yield a triazole carboxylic acid metabolite. Both ribavirin and its triazole carboxylic acid metabolite have renal excretion.

The long washout half-life of ribavirin reflects extensive accumulation in all tissue compartments, including red blood cells, and slow clearance from these compartments. For this reason, ribavirin steady-state concentrations are not reached until week 4 of treatment. Finally, the kinetics of ribavirin is influenced by food; administration in the fed state increases the competition phenomena between some nucleoside analogues to reach appropriate use of the drug.

Clearance (CL) and volume of distribution (V) are the most important factors. With respect to V ribavirin, it is mainly a function of body weight. In contrast, CL ribavirin depends on body weight and creatinine clearance (CLCR). As ribavirin is mainly cleared by the kidneys, the renal function has a large influence on CL ribavirin. In studies enrolling patients with wide ranges of renal function parameters, CLCR predicted CL ribavirin better than body weight.

Population pharmacokinetic studies have examined which factors influence the variability of ribavirin pharmacokinetics. Clearance (CL) and volume of distribution (V) are the most important factors. With respect to V ribavirin, it is mainly a function of body weight. In contrast, CL ribavirin depends on body weight and creatinine clearance (CLCR). As ribavirin is mainly cleared by the kidneys, the renal function has a large influence on CL ribavirin. In studies enrolling patients with wide ranges of renal function parameters, CLCR predicted CL ribavirin better than body weight.

Donnerer et al. found a significant negative correlation between ribavirin plasma levels and glomerular filtration, suggesting that ribavirin exposure could be mainly determined by the glomerular filtration rate. However, in other studies conducted in patients with normal renal function, body weight was the variable with the largest influence on CL ribavirin. Thus, the influence of CLCR on CL ribavirin could be more important when renal function is impaired, specifically only when CLCR falls <43 mL/min.
In HIV patients with chronic hepatitis C, who are at higher risk for renal diseases including HIV-associated nephropathy and damage caused by antiretroviral drugs (e.g., indinavir, tenofovir etc.), this consideration could be particularly relevant.

The variability in ribavirin pharmacokinetics among individuals may be influenced by urine pH. As ribavirin is an acid compound and is excreted in urine without any change in its molecular structure, acidification of urine might increase ribavirin renal re-absorption and thus increase serum ribavirin levels. Accordingly, Arase et al. found that patients were more likely to achieve high ribavirin exposures when the average urine pH was below 6. Thus, it would be important to monitor in parallel serum ribavirin concentrations and urine pH.

Despite all prior considerations, a large proportion of variability in CL ribavirin (ranging from 40% to 73%) cannot be explained by variables such as gender, age, CL-CR and body weight. This means that other not yet identified parameters may influence ribavirin pharmacokinetics. This consideration may further support TDM for ribavirin at an individual level.

**Monitoring ribavirin plasma levels**

Drugs have to fit some criteria for being good candidates for TDM, including clear pharmacokinetic information, high inter-patient and low intra-patient variability in plasma concentrations, close correlation between plasma drug concentrations and drug activity as well as incidence of side effects and narrow therapeutic range. Ribavirin shows a wide inter-individual variability with low intra-individual variations in plasma ribavirin concentrations (25% to 30%), which supports TDM. As ribavirin is converted into its active triphosphate metabolite within cells, plasma or serum levels may not be a good predictor of intracellular triphosphate concentrations. Although it is not feasible to measure the concentration of intracellular triphosphate analogues in hepatocytes, there are methods available to measure ribavirin concentrations within erythrocytes. However, they are more complicated than those required for measuring ribavirin in serum or plasma. Despite all these objections, almost all studies have demonstrated a good pharmacokinetic-pharmacodynamic relationship between ribavirin concentrations and therapeutic response and toxic effects, which supports TDM for ribavirin. Finally, the samples employed for the quantification of ribavirin levels can be serum, plasma or peripheral blood (for intra-erythrocyte ribavirin). Different parameters reflecting the exposure to ribavirin can be measured following one interval dose: the most frequently used is the minimal concentration obtained just before intake of the next dose ($C_{\text{min}}$); $C_{\text{max}}$ is the maximal concentration achieved in the dose interval and AUC is a measure of global exposure of the drug. The methodology used to quantify ribavirin levels is HPLC or HPLC–tandem mass spectrometry (HPLC/MS) after solid-phase extraction by distinct well-established protocols.

So far, a total of 20 studies of relevant data on TDM of ribavirin have been performed. Most of them were performed in HCV mono-infected patients (13), 5 were in HIV/HCV co-infected patients, 1 in HIV patients in haemodialysis and 1 in HCV patients with liver transplantation. Table S1 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/) summarizes the main characteristics of studies that have explored the relationship between ribavirin concentrations in plasma, serum or erythrocytes and pharmacodynamic variables associated with toxicity (e.g. anaemia) and/or efficacy (e.g. EVR or SVR).

**Correlation between ribavirin plasma concentration and virological response**

**Ribavirin exposure and EVR**

Only two studies so far did not find a correlation between ribavirin plasma levels and HCV-RNA suppression at week 12. The rest found a good correlation and defined a target ribavirin concentration at week 4 that predicted EVR (>2 log drop in serum HCV-RNA at week 12 of therapy). In HCV mono-infected patients, Souvignet et al. found that a threshold of 2.17 mg/L was reached by half of the patients who achieved EVR. In HIV/HCV co-infected patients, a threshold of 2.7 mg/L at week 4 was proposed by Rendón et al. giving a sensitivity of 70% and a specificity of 49%. A lower cut-off was found by Aguilar Marucco et al. of 1.6 mg/L of ribavirin concentration through treatment (weeks 2–48) to discriminate EVR for HCV genotype 1 or 4, with a sensitivity of 88.9% and a specificity of 63.7%. Finally, Domínguez et al. have recently found a ribavirin concentration threshold of 1.95 mg/L for HCV genotypes 1 and 4, with a sensitivity of 75%. The same authors reported a threshold of 146 mg/L for erythrocyte ribavirin concentration able to predict EVR, regardless of HCV genotype.

**Ribavirin exposure and SVR**

Persistently negative serum HCV-RNA beyond 24 weeks upon completion of HCV therapy defines SVR. All studies that have assessed a relationship between ribavirin plasma concentrations and SVR found an association. Jen et al. found an SVR rate of 49% when ribavirin plasma concentrations at week 4 were 3.5–4 mg/L. This figure increased to 62.5% when ribavirin plasma concentrations were >4 mg/L. The same authors noted that HCV genotype 1 required higher ribavirin plasma concentrations than genotypes 2 and 3 to reach the same response rate. Based in part on these results, Lindahl et al. performed a prospective study in 10 patients infected with HCV genotype 1 to evaluate the safety and tolerance of a high dose of ribavirin that was selected and adjusted to achieve a steady-state ribavirin concentration of ≥3.66 mg/L. Nine of the 10 patients achieved SVR, despite the fact that side effects were more frequent and serious than using standard ribavirin doses. Tsubota et al. found an SVR rate of 100% in patients infected with HCV-1b when ribavirin plasma concentrations were 2.5–3 and >3 mg/L at weeks 4 and 8, respectively. Similar results were obtained by Arase et al. and Maynard et al. at weeks 8 and 4, respectively. Interestingly, Loustaud-Ratti et al. evaluated the relationship between early ribavirin exposure and virological response and found that the area under the curve interdose (AUC$_{0-12}$) and an abbreviated AUC$_{0-4}$ after the first dose of ribavirin, but not the AUC at week 12, were correlated with SVR. Two cut-offs were established for AUC$_{0-12}$ and AUC$_{0-4}$ of 3014 μg/h/L with a sensitivity of 91% and a specificity of 61% and 1755 μg/h/L with a sensitivity of 72% and a specificity of 85%, respectively.

In HIV/HCV co-infected patients, Rendón et al. examined the targeted ribavirin plasma concentration through treatment...
(weeks 4–48) associated with SVR. Levels >2.79 mg/L predicted overall SVR with 81% sensitivity and 52% specificity. These figures were 72% and 65% for patients infected with HCV genotype 1 or 4 using a higher threshold of 2.86 mg/L, respectively. In contrast, Aguilar Marucco et al. established a lower cut-off of 1.6 of ribavirin concentration through treatment (weeks 2–48) to discriminate SVR for HCV genotype 1 or 4 with 87.5% sensitivity and 58.3% specificity only for genotypes 1 and 4. In a recent study, Domínguez et al. established a threshold of 1.95 mg/L for plasma levels with 58% sensitivity and 146 mg/L for erythrocyte ribavirin concentrations with 75% sensitivity at week 4 only for genotypes 1 and 4, respectively. Finally, Dahari et al. evaluated the role of early ribavirin pharmacokinetics in predicting SVR in 24 co-infected patients. Surprisingly, ribavirin plasma levels during the first 2 weeks were lower in patients with SVR than in non-responders, but the rate of ribavirin accumulation between weeks 2 and 8 (when steady state was achieved) increased significantly in patients with SVRs and only modestly in non-responders. In contrast, Hb decline during the first month of therapy was greater in patients with SVR than in non-responders.

In patients after liver transplantation, Dumortier et al. compared the efficacy, safety and ribavirin plasma levels when using increasing dosage of both ribavirin and pegylated interferon α-2b with a control cohort of non-transplanted patients. Mean ribavirin plasma levels, virological response rates and tolerance were similar in the two groups, although higher maximum Hb decline was reached in the liver transplantation group than in the control group.

In patients with renal dysfunction, Van Leusen et al. evaluated the efficacy and tolerability of a strategy to adjust ribavirin plasma levels to a defined therapeutic range of 1.5–2.5 mg/L in seven patients with end-stage renal disease. SVR was achieved by 75% of the patients. None of the patients discontinued the study due to adverse side effects, but reductions in ribavirin and pegylated interferon α-2b doses, and increases in erythropoietin doses and blood transfusions were required in some patients.

Correlation between ribavirin plasma concentration and anaemia

Only one study so far has not found a correlation between ribavirin plasma or serum levels and the extent of Hb decline compared with baseline. In the rest, a linear correlation between ribavirin plasma levels and the extent of Hb decline was seen. Maeda et al. found that Hb drops below 8.5 g/dL mainly occurred when ribavirin concentrations were >3.5 mg/L. Likewise, Arase et al. found that serum ribavirin concentrations >3.5 mg/L were associated with a higher incidence of treatment dropouts due to anaemia. In another study, Uchida et al. found that patients who discontinued HCV medications during the first 4 weeks of treatment due to anaemia had higher ribavirin plasma levels at week 1 than patients who tolerated the same treatment. Finally, Loustaud-Ratti et al. found a correlation between Hb levels at week 12 and AUC values after the first dose of ribavirin but not at week 12.

In HIV/HCV co-infected patients, Rendón et al. found that the only variables independently associated with higher Hb declines were greater ribavirin plasma concentrations and concomitant zidovudine use. Ribavirin plasma concentrations >2.8 mg/L could predict Hb drops >2 g/dL at weeks 4 and 12 with a sensitivity of 73% and a specificity of 81%. Similar results were obtained by Souvignet et al., who found that the ribavirin serum concentration that gave >2 g/dL drop in Hb in 50% of the patients was 2.44 mg/L. A lower cut-off of 2.3 mg/L was established by Aguilar Marucco et al. for ribavirin concentration through treatment (weeks 2–48) associated with an Hb decrease of >4 g/dL with a sensitivity of 46.2% and a specificity of 89.7%.

As previously mentioned, Inoue et al. did not find a correlation between ribavirin plasma levels and Hb reductions. However, a significant correlation was found between Hb drops and ribavirin concentrations within erythrocytes 14–28 days after starting combination therapy for HCV. Erythrocyte ribavirin concentrations >244.2 mg/L predicted a greater Hb drop. Similar results highlighting the correlation between erythrocyte ribavirin concentrations and the extent of Hb decline have been reported by others, including one study which assessed HIV/HCV co-infected patients.

Summary

Several attempts have been made to predict EVR, SVR and anaemia on the basis of ribavirin plasma trough concentrations with variable success. Differences between study results may be explained by distinct characteristics of the targeted patient populations, interferon and ribavirin therapies and employed methodologies for the quantification of ribavirin levels. First, characteristics of patients such as age, CLCR, gender, body weight and race may affect ribavirin pharmacokinetics. Moreover, enough exposure to ribavirin could be more relevant for achieving SVR in patients with HCV genotype 1 or 4 than 2 or 3. Secondly, differences in type and/or dose of interferon and ribavirin between studies may also explain the differences in proposed thresholds. Pegylated interferon α versus standard interferon and pegylated interferon α-2a versus α-2b have demonstrated higher antiviral activity. In fact, almost all studies performed with standard interferon found higher cut-offs. Use of concomitant drugs (e.g. antiretrovirals) may also affect pharmacokinetic results. Finally, a rigorous methodology is necessary to assess ribavirin levels correctly. Since there are no quality control programmes to validate results produced using different tests, inter-assay variability might also account for some discrepancies. Other aspects such as the methodology of blood sample processing in order to obtain the plasma or serum is very important due the haemolysis of red blood cells that can overestimate ribavirin exposure. The choice of plasma instead of serum is also important. Plasma ribavirin concentrations have been shown to be more reproducible than serum intensive-sampling pharmacokinetic studies. Despite all these limitations, almost all studies have demonstrated that ribavirin plasma, serum or erythrocyte levels correlate with the development of anaemia and with the achievement of either EVR or SVR. Therefore, early TDM might help to tailor ribavirin dosages, improving efficacy and safety of anti-HCV treatment.

Three specific questions have been addressed in this review. First, which should be the preferred initial dose of ribavirin? Since CL ribavirin is strongly dependent on renal function, any adjustment of ribavirin doses should be based on body weight and on the degree, if any, of impaired renal function.
Second, when should ribavirin plasma concentrations be monitored? As early decline in serum HCV-RNA after initiation of anti-HCV therapy seems to be crucial for the final outcome, the sooner ribavirin is monitored the earlier we may adjust ribavirin doses in an attempt to optimize drug exposure. Accordingly, assessment at week 4, once steady-state has been reached, may be proposed as the best time to monitor ribavirin plasma concentrations. However, some studies have proposed an earlier monitoring of ribavirin concentrations (before week 4). It has been suggested that differences in ribavirin pharmacokinetics between responders and non-responders appear mainly during the first week since early ribavirin exposure might enhance the response of interferon-stimulated genes. More studies are required to confirm these findings because the measurement of ribavirin concentration in plasma before the steady state may not be representative of the concentration at the target site.

Third, what is the therapeutic range for ribavirin? The chances of response increase with greater ribavirin plasma concentrations. According to all studies reviewed, we propose a target concentration of at least 2–2.5 mg/L to maximize the achievement of SVR. Unfortunately, ribavirin plasma concentrations >2.5 mg/L are associated with significant rates of haemolytic anaemia. Therefore, the severity of anaemia will finally dictate the possibility of adjusting ribavirin dose to the proposed threshold. Adjustment of ribavirin dosage should be performed using pharmacokinetic models. However, since pharmacokinetic models are not usually available in the clinical setting, we propose to adjust ribavirin dosage empirically with subsequent monitoring drug concentrations to assure an adequate exposure.

In conclusion, TDM for ribavirin could be especially beneficial for specific populations such as patients with significant drug–drug or drug–food interactions, individuals with impaired gastrointestinal, hepatic or renal function, and pregnant women or paediatric patients. Special attention has to be given to patients infected with HCV genotype 1 or 4, or co-infected with HIV, in whom the effectiveness of pegylated interferon α is lower. Randomized studies and controlled clinical trials should be performed to prove the benefit of dose adjustments based on early TDM for ribavirin.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


