A Pharmacological Profile of Ribavirin and Monitoring of its Plasma Concentration in Chronic Hepatitis C Infection

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Chronic hepatitis C (CHC) infection, usually an asymptomatic infection, has long-term serious complications such as cirrhosis, hepatocellular carcinoma, and end-stage liver disease requiring liver transplantation (LT). Several novel drugs against hepatitis C which form part of 'specifically targeted antiviral therapy for hepatitis C' (STAT-C) have been developed. These include NS3/4A protease inhibitors telaprevir, boceprevir, and nucleoside/non-nucleoside polymerase inhibitors (NS5A) which hold promise for future therapy. Despite the development of new anti-hepatitis C virus (HCV) drugs, ribavirin (RBV) remains the single most important drug to prevent relapse and is frequently included among newer regimens being developed with novel small molecule anti-HCV drugs. The current approved treatment is a combination therapy of once weekly subcutaneous pegylated-interferon (PEG-IFN)- α plus body-weight-based oral RBV regimen. The most significant dose-dependent side effect of RBV is hemolytic anemia warranting dose reduction or discontinuation in severe cases compromising sustained virological response (SVR). Monitoring RBV plasma concentration has been challenging due to its peculiar pharmacokinetics and has been done to predict both efficacy and toxicity. Herein, we review the pharmacological profile of RBV and the monitoring of its plasma concentration, monitoring in renal impairment, post-LT, and human immunodeficiency virus (HIV)-HCV co-infection in patients being treated with combination therapy of PEG-IFN- α and RBV. (J CLIN EXP HEPATOL 2012;2:42–54)

Hepatitis C virus (HCV), a ribonucleic acid (RNA) virus, affects about 3% of the world's population with prevalence of chronic hepatitis C (CHC) infection being highest in Egypt (>15%) followed by other African countries (<15%). Prevalence in most other countries is <3% including India (0.9%).^{1–3} An estimated 4.1 million people are affected by HCV infection in the United States of America (1.6%).⁴ Hepatitis C virus infection results in chronicity in 65–85% cases with long-term

complications such as progressive liver fibrosis, cirrhosis, hepatocellular carcinoma evolving over several years, and end-stage liver failure warranting liver transplantation (LT).⁵ Treatment response varies depending on the genotype which is of six major types (and >80 subtypes). Genotype 1 predominates in the USA,⁶ while genotype 3 predominates in India.^{7,8} Genotype 4 is common in Egypt and other Middle East countries while genotype 6 is seen in Vietnam and other Southeast Asian countries.⁹ The best pre-treatment predictor of response to anti-HCV medications seems to be the HCV genotype, which also dictates the duration of treatment. Genotypes 2 and 3 respond to combination treatment of pegylated-interferon (PEG-IFN)- α and ribavirin (RBV) in a favorable way with sustained virological response (SVR) rates up to 80% when treated for 24 weeks, dose of RBV being 800 mg irrespective of weight.¹⁰ However, SVR achieved with the same combination therapy for genotype 1 is <50% with 48 weeks treatment, dose of RBV being 1000 mg for weight <75 Kg and 1200 mg for weight >75 Kg,¹¹ probably contributing to higher incidence of adverse effects among these patients. The dose of PEG-IFN- α -2a is 180 μ g/week (subcutaneous) and 1.5 μ g/Kg for PEG-IFN- α -2b.¹² Another Food and Drug Administration (FDA)-approved combination therapy includes boceprevir/telaprevir (protease inhibitors) as add-on treatment to PEG-IFN- α plus oral RBV. Several directly acting antiviral agents (DAAs) are in late stage of clinical development at present.¹³ In India, combination

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Abbreviations: ADSS: adenylosuccinate synthetase; ATP: adenosine-5'-triphosphate; AUC: area under concentration curve; BMD: bone mineral density; C_{ss} : concentration at steady-state; C_{trough} : trough concentration; DAAs: directly acting antiviral agents; EPO: erythropoietin; EVR: early virological response; FDA: Food and Drug Administration; GTP: guanosine-5'-triphosphate; Hb: hemoglobin; HCV: hepatitis C virus; HIV: human immunodeficiency virus; HPLC: high-performance liquid chromatography; IFN: interferon; IMPDH: inosine-5'-monophosphate dehydrogenase; ITPA: inosine triphosphate pyro-phosphatase; PEG-IFN: pegylated-interferon; PSH: protein sulphydryl; RBC: red blood cell; RBV: ribavirin; RNA: ribonucleic acid; RSV: respiratory syncytial virus; RVR: rapid virological response; STAT-C: specifically targeted therapy for hepatitis C; SVR: sustained virological response; TDM: therapeutic drug monitoring

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treatment of PEG-IFN- α and oral RBV is approved while the new protease inhibitors as add-on treatment to the existing regimen are yet to be available.

The most significant side effect of RBV is dose-dependent hemolytic anemia warranting dose reduction or even discontinuation in severe cases. Fall in hemoglobin (Hb) due to RBV is accentuated by PEG-IFN- α on account of bone marrow suppression. Sustained virological response is said to be achieved when HCV-RNA levels are undetectable 24 weeks after successful completion of therapy. The CHC infection is theoretically amenable to cure as HCV does not integrate into the host cell genome. Despite the development of novel anti-HCV drugs, RBV remains the single most important drug to prevent relapse¹⁴ and is likely to remain in combination therapy with newer agents.¹⁵ Therapeutic drug monitoring (TDM) may be helpful to achieve RBV levels which improve SVR rates as well as limit hematological side effects. Ribavirin plasma concentration has been measured to predict both efficacy and toxicity. However, the peculiar pharmacokinetics of RBV has eluded researchers as to the precise time to measure RBV on account of late attainment of steady-state concentration (usually 4–8 weeks). Several studies have shown correlation of plasma concentration of RBV with efficacy and toxicity. However, the optimal therapeutic concentration range is still inconclusive. Studies have either supported or refuted TDM. This review article emphasizes on the pharmacological profile of RBV in HCV and utility of monitoring its plasma concentration, monitoring in renal impairment, post-LT, human immunodeficiency virus (HIV)-HCV co-infection in patients being treated with combination therapy of PEG-IFN- α and RBV.

Mechanism of Action of Ribavirin and Clinical Efficacy in Hepatitis C Virus Infection

Since, the discovery of RBV in 1970, several mechanisms have been proposed to explain its antiviral activity. The RBV (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide; Figure 1), a guanosine analog after intracellular phosphorylation by host cell enzymes to phosphorylated metabolites namely RBV 5'-monophosphate and 5'-triphosphate, exerts antiviral

Figure 1 Structure of ribavirin.

activity predominantly via inhibition of cellular inosine 5'-monophosphate dehydrogenase (IMPDH) activity which results in depletion of intracellular guanosine-5'-triphosphate (GTP).¹⁶ Other mechanisms that have been proposed include inhibition of viral RNA-dependent polymerase/NS5B (by RBV 5'-monophosphate), inhibition of capping of viral mRNA (by RBV 5'-triphosphate), and induction of mutagenesis in HCV genome (on account of incorporation into viral genome).^{16,17} Atsukawa et al¹⁸ have recently shown that RBV polarizes the Th1/2 (T-helper cell) balance to Th1 predominance; down-modulates induced co-stimulator (inducible co-stimulator [ICOS], B7-H2 ligand on dendritic cells which helps in differentiating Th0 to Th2 cells) and reduces IL-10 production from CD4+ T-cells (exposed to RBV).

Ribavirin causes upregulation of IFN-stimulating genes thereby potentiating the effect of IFN- α in combination treatment. Ribavirin monotherapy has been shown to be inferior to IFN monotherapy and as such not recommended.¹⁹ Addition of RBV to IFN accelerates the second slope of viral decay thereby reducing the incidence of relapse.²⁰ Ribavirin when combined with protease inhibitors (telaprevir/boceprevir) has shown additive antiviral effect. Triple therapy of telaprevir, RBV, and PEG-IFN- α has shown a more superior synergistic activity than with dual therapy. The impact of RBV in such a treatment regimen is by preventing viral breakthrough and reducing the risk of selecting for resistance to DAAs.²¹ Several randomized trials have proven clinical efficacy of RBV in combination treatment with IFN- α by increasing rates of SVR and reducing relapse. Indeed, it has remained the most important agent to prevent relapse; hence is frequently included in regimens involving newer DAAs. It has shown synergistic antiviral activity even in IFN-free regimens. PROVE II and III trials have demonstrated the impact RBV has in combination treatment of HCV (genotype 1). Higher rates of SVR, reduced viral breakthrough, and relapse were noted in regimens involving RBV along with telaprevir and IFN. Control groups without RBV showed lesser SVR rates with higher relapse rates and viral breakthrough.^{22,23} The HCV SPRINT-1²⁴ study assessed boceprevir with IFN- α and RBV in genotype 1 HCV patients with varying doses and showed lesser SVR rates in the low-dose RBV group with higher rates of viral breakthroughs. Thus, it is clear that RBV is critical in combination treatment whether it is an IFN-based regimen or a protease inhibitor regimen or a triple regimen involving both.

Adverse Effects of Ribavirin

Side effects of RBV are generally less severe than IFN. The most distinctive side effect of RBV is dose-dependent hemolytic anemia and is the most frequent side effect necessitating dose reduction or discontinuation of therapy (dose reductions in up to 39%; discontinuation up to 16%

of patients on combination treatment) in severe cases.^{25–28} Decline in Hb level typically begins within few days of initiation of therapy, maximum decline occurring within 4 weeks. Anemia-associated with RBV is reversible as evident from Figure 2. Stabilization occurs following peak fall in Hb levels with eventual recovery upon completion of treatment or discontinuation likewise. Hemoglobin levels should hence be measured at baseline, weeks 2 and 4 and thereafter as needed.^{26,28} It has been shown that dose reduction amounting to patients receiving <60% of the original dose leads to poor rates of SVR, stressing the importance of continuing RBV till the end of therapy by careful management of side effects, as even late discontinuation can jeopardize treatment success.¹⁵ Anemia is more troublesome in those with underlying cardiovascular disease and screening for cardiac disease should be done prior to initiating RBV.²⁶ The other side effects are less severe and are tolerable in most cases. Nausea has been noted in more than one-fourth of patients receiving RBV.²⁹ Combination therapy has higher incidence of dermatological side effects and includes generalized pruritus, eczematous lesions (extremities), and dry skin.³⁰ Teratogenicity of RBV is of significant concern and hence is contraindicated in pregnancy and male partners of pregnant women, the drug being included in pregnancy category X (FDA).²⁸

Teratogenicity has been consistently shown in animal studies (at one-twentieth recommended human dose; malformations of the skull, eye, jaw, limbs, etc.) and hence is presumed to be toxic in humans and pregnancy should

be avoided during the entire treatment period as well as 6 months following cessation of treatment.^{28,31,32} Extreme caution must be exercised before using RBV in renal impairment. Bone mineral density (BMD) loss has also been reported with RBV use but is uncommon.³³ Rigors occur when RBV is given as intravenous (i.v.) bolus injection (for non-HCV indications).³⁴ Aerosolized RBV commonly used for respiratory syncytial virus (RSV) infection can cause respiratory side effects such as bronchospasm, hypoxia, pulmonary edema, bacterial pneumonia, and ventilator dependence. Healthcare workers exposed to aerosolized RBV can also develop respiratory side effects such as bronchospasm and pharyngitis particularly in those with underlying airway disease.^{28,35}

Mechanism of Ribavirin-induced Anemia and Risk Factors

Ribavirin is transported from the plasma to red blood cells (RBCs) through the equilibrative nucleoside transporter 1 and undergoes phosphorylation to form RBV 5'-triphosphate. Lack of 5'-nucleotidase and alkaline phosphatase in RBCs leads to accumulation of phosphorylated forms of RBV. Depletion of GTP, inhibition of adenylosuccinate synthetase (ADSS), and consequent adenosine-5'-triphosphate (ATP) deficiency primarily contribute to RBV-induced anemia.^{36,37} Relative ATP deficiency in RBCs damages the oxidative stress defense mechanism (indirectly), due to reduced formation of glucose-6-phosphate. Tanaka et al³⁸ showed that changes in serum and RBC membrane lipids contribute to reduced deformability and fluidity leading to hemolysis. However, the relative importance of RBC membrane lipid changes in the pathogenesis of RBV-induced hemolysis has been questioned.³⁹ Hitomi et al³⁷ have recently demonstrated that inosine triphosphate (ITP) protects against RBV-induced hemolytic anemia by substituting for erythrocyte GTP in the biosynthesis of ATP. Increase in ITP levels as seen in ITP pyro-phosphatase (ITPA) variants due to reduced ITPA function (individuals with low activity mutations in ITPA gene) was shown to have far lesser depletion of ATP than individuals with normal ITPA suggesting usefulness of modulation of ITP to prevent RBV-induced anemia in future. However, it was clear from the study that ITP itself cannot replace ATP. Although functional variants of the ITPA gene on chromosome 20 causing ITP deficiency have been shown to protect against RBV-induced anemia and counter-dose reductions, the same result has not been shown in genotype 2 and 3 infected individuals.

Other mechanisms that have been proposed to explain anemia-induced by RBV include reduced membrane bound IgG, C3, and erythrocyte band 3.³⁶ Red blood cell-related risk factors such as glutathione, thioredoxin, and membrane protein alterations have been shown to predict RBV-induced anemia.⁴⁰ Several studies have identified the

Figure 2 Graphical representation of decline in hemoglobin (Hb) levels during combination treatment of ribavirin (RBV) and interferon (IFN)-α (standard doses) for 24 weeks in genotype 2 and 3 patients. Fall in Hb occurs early in the treatment as shown in the illustration. Maximum decline occurs by the 4th week followed by stabilization phase and normalization upon completion of therapy. However, high RBV concentration >3.5 μg/mL leads to severe anemia (<10 g/dL; >4g/dL decline), and Hb levels should be preferably maintained below this level. This illustration emphasizes the need for early monitoring to limit hematological side effects. The fall is steeper in the presence of co-medications such as zidovudine, human immunodeficiency virus-hepatitis C virus co-infection, and renal dysfunction.

risk factors for developing severe anemia. Reau et al⁴¹ noted that renal impairment and fall in Hb ≥ 1.5 g/dL at week 2 of treatment were good early predictors of substantial fall in Hb during the later course of treatment (≥ 2.5 g/dL at week 4). Hung et al⁴² in 490 Taiwanese HCV patients assessed several possible risk factors for developing severe anemia and showed that old age, female gender, baseline Hb, low body weight, and pre-treatment platelet counts were independent risk factors for developing severe anemia. Van Vlierberghe et al⁴³ observed that pre-treatment higher Hb levels resulted in higher fall in Hb; lower pre-treatment platelet count and haptoglobin phenotype were found to be additional significant risk factors for developing severe RBV-induced anemia. It has also been shown that lower pre-treatment protein sulfhydryl (PSH) values increase risk for developing severe RBV-induced hemolysis.⁴⁴

Pharmacokinetics of Ribavirin

Ribavirin is absorbed orally and widely distributed in tissues including cerebrospinal fluid (slow distribution) attaining about 70% of plasma concentrations. Bioavailability is 45–65% on account of first-pass metabolism. Bioavailability of RBV increases with fatty meal (*F* increases to about 70%) and is recommended to be taken with food. Volume of distribution (V_d) is very high, about 4500–5000 L. It has no plasma protein binding. It accumulates extensively in the plasma in a way that C_{max} at steady-state is more than 4-fold the C_{max} following single dose.⁴⁵ Preston et al⁴⁶ in 6 health volunteers have reported a mean C_{max} following single 150 mg i.v. dose and 400 mg oral dose to be 4187 ng/mL and 638 ng/mL, respectively, with a mean difference of 3549 ng/mL. Ribavirin has slow elimination (prolonged terminal clearance) from compartments other than plasma (RBCs and other tissue compartments; the exit $t_{1/2}$ of RBV in erythrocytes is about 6 weeks). Ribavirin has a long half-life (120–170 hr following a single dose; ~300 hr after multiple dosing).²⁸ Following 600 mg BD dosing, steady-state has been shown to attain at 4 weeks. However, steady-state may not be attained until 11 weeks (usually attains after 4–6 half-lives) on account of long wash-out half-life owing to slow clearance from RBCs and other tissue compartments.^{32,45,47} Ribavirin is neither an inducer nor an inhibitor of hepatic CYP 450 enzymes. It is metabolized possibly by two pathways: reversible phosphorylation pathway or pathway involving deribosylation and amide hydrolysis (to a triazole carboxylic acid derivative). It is transported from plasma to non-plasma compartments (especially RBCs and other cells in tissue compartment) by nucleoside transporters which include hCNT2, hCNT3, hENT1, and hENT2.^{48,49}

No pharmacokinetic interaction has been seen with concurrent use of RBV and PEG-IFN- α . Ribavirin (and its metabolites) is cleared by the kidneys and hence accumulates in renal impairment and dialysis does not significantly remove RBV as its volume of distribution is several

thousand liters. Pharmacokinetics of RBV is not altered with hepatic impairment; so no dose adjustment of RBV is required. Age does not seem to contribute significantly to variation in RBV kinetics though elderly (> 65 Kg) should be treated with careful attention to renal function.³² Wade et al⁴⁷ developed a three-compartment model to describe the kinetics of RBV with a sequential zero-order and a first-order absorption process. Fatty meal increased the relative bioavailability by a factor of 1.46. Among the various co-variables assessed for possible influence on kinetics of RBV, only race and lean body weight (41–95 Kg) had statistical influence but clinically significant influence on kinetics (affecting clearance and volume of the peripheral compartment) was seen only with lean body weight. The model predicted variation of clearance to be between 15.3 L h^{-1} and 23.9 L h^{-1} over 41–95 Kg lean body weight. Also, clearance of RBV is dependent on both body weight and creatinine clearance. Thus, in renal impairment, influence of creatinine clearance on RBV clearance is important (more so when Cl_{cr} is $< 43 \text{ mL/min}$). High interindividual variability of RBV with lesser intra-individual variability ($< 25\%$ for area under concentration curve [AUC] and C_{max})³² in kinetics, correlation between plasma concentration and efficacy/toxicity, alteration of kinetics in renal impairment, compromise in achieving SVR upon significant dose reduction, and narrow therapeutic index make it a suitable candidate for TDM. However, considerable confusion prevails with respect to therapeutic levels required to achieve SVR without causing unacceptable toxicity.

What Time Point and Specimen to Choose for Measuring Ribavirin Levels?

Different time points have been used to measure RBV concentration. Trough concentration (C_{trough}) has been most commonly used, the concentration of RBV measured just before administering the next dose; C_{max} has been less frequently used in TDM of RBV, the maximum concentration reached in a dose interval.⁵⁰ Loustaud-Ratti et al⁵¹ for the first time used $\text{AUC}_{0-12 \text{ h}}$ (area under the curve representing total exposure of RBV in a dose interval) and $\text{AUC}_{0-4 \text{ h}}$ to demonstrate correlation between early global exposure of RBV and SVR. However, C_{trough} seems to be the most convenient and suitable time point to use for measuring RBV levels. The method commonly employed to measure RBV is high-performance liquid chromatography (HPLC) using solid-phase extraction by well-defined protocols.⁵² LC-MS/MS has also been employed to accurately estimate RBV in plasma.⁵³

Although, RBV concentrates in RBCs and hepatocytes as triphosphate metabolites, plasma concentration measurement has shown good relationship between kinetics and dynamics and is more consistent than serum measurements.⁵⁴ Marquet et al⁵⁵ have reported higher inter-laboratory variations in concentration of RBV in serum

than in plasma. To measure RBV levels in hepatocytes is impractical, but erythrocyte measurements are possible though complex. Employment of intracellular monitoring of RBV on account of being good predictors of anemia and efficacy cannot be employed routinely on account of complexity and cost of such measurements.

When to Measure Ribavirin Levels?

This question has eluded researchers on account of late achievement of steady-state concentration of RBV (varies between 4 weeks and 11 weeks). Several studies have measured RBV plasma concentration at week 4 as many patients achieve steady-state concentration once week 4 is reached. Recent studies have shown that steady-state may not reach until week 8. Weeks 4, 8, and 12 have been commonly used to measure RBV levels.^{56,57} Measuring RBV concentration to correlate for efficacy/toxicity before achievement of steady-state concentration may not be the actual representative concentration, leading to inaccurate interpretations. However, attempts have been made to measure RBV concentration early in the treatment to predict steady-state concentration.

Slavenburg et al⁵⁸ have studied RBV plasma trough concentrations at weeks 1, 2, 4, 8, 12, 16, 20, and 24 to see for correlation between early (weeks 1 and 2) and steady-state concentrations, time at which steady concentration is reached, interpatient and inpatient variability seen with RBV dosed on an oral weight-based regimen (12–15 mg/Kg). They showed that steady-state concentrations were achieved only by the 8th week and that early concentrations correlated with steady-state concentrations. The interpatient and inpatient variability were 43% and 13%, respectively, following 8 weeks of therapy. This study encouraged early RBV monitoring (as early as 2 weeks) as decline in Hb starts quite early in the treatment. Early decline in HCV-RNA is also critical as far as final outcome is concerned. Prediction of steady-state concentrations based on early measurements of RBV to foresee the extent of Hb decline very early in the treatment allows clinicians to adjust/individualize dose of RBV to prevent/reduce treatment-related side effects, at the same time maintain adequate exposure of RBV to achieve SVR. More prospective studies are needed showing consistent correlation between early and steady-state concentrations of RBV (thus helping in predicting concentration at steady-state [C_{ss}]) before employing early monitoring of RBV.

Correlation of Plasma/Serum Concentration of Ribavirin with Sustained Virological Response: Evidence for and Against it

Several studies have shown a definite relationship between RBV plasma/serum concentration and SVR/early virological response (EVR). Higher level of RBV increases the chance of achieving SVR, at the cost of hematological

side effects. Arase et al⁵⁹ reported in HCV genotype 1b Japanese patients on combination therapy of PEG-IFN- α -2b and RBV, higher rates of SVR when serum levels of RBV exceeded 3 μ g/mL at week 8 (SVR of 25% overall vs 44% in those with levels >3 μ g/mL). This parameter was shown in univariate analysis to significantly predict the achievement of SVR. However, levels exceeding 3.53 μ g/mL at week 8 resulted in significantly higher ($P=0.033$) dropouts/dose reductions and hence they proposed a therapeutic range of 3–3.5 μ g/mL at week 8 of combination therapy to achieve SVR and limit side effect-related dose reductions. Similarly, Tsubota et al⁶⁰ in a pharmacokinetic study noted higher steady-state serum RBV levels in patients who achieved SVR in comparison to non-responders.

Lindahl et al⁶¹ assessed the safety and tolerability of high-dose RBV along with standard doses of PEG-IFN- α -2a in 10 genotype 1 HCV patients treated for 48 weeks. Dosing methods used in the study was an initial calculated dose based on renal function using a kinetic formula. Subsequently based on levels achieved during monitoring, dose adjustments were made in order to achieve steady-state of >3.66 mg/L. Ninety percent of patients achieved SVR using such an individualized dosing strategy though the incidence of hematological side effects among these patients was high. Erythropoietin (EPO) and blood transfusions were also used to combat anemia in addition to dose reductions of RBV. Maynard et al⁶² proposed a cutoff level of RBV at week 4 to be 2 mg/L to achieve higher rates of SVR. They found in 35 HCV (genotype 1) patients, plasma concentrations above 2 mg/L at week 4 achieved 67% SVR rate while achieving only 16% in those with levels <2 mg/L. This parameter was shown in multivariate analysis to predict achievement of SVR independently. Loustaud-Ratti et al⁵¹ evaluated the relationship between early global exposure of RBV and SVR in genotype 1 HCV patients. They found good correlation of early RBV exposure (Day 0 AUC_{0–12h} and AUC_{0–4h}) with SVR, wherein higher levels were noted in those who achieved SVR. Based on the above findings, they proposed a minimum threshold of 1755 μ g/h/L (AUC_{0–4h}) for RBV on day 0 of treatment.

Saito et al,⁶³ however, failed to show any correlation between serum concentration of RBV and SVR. Pederson et al⁵⁶ in a randomized controlled trial assessed correlation of plasma trough concentration of RBV with SVR at day 29 and week 12 of treatment among genotype 2 and 3 HCV-infected patients receiving combination therapy of PEG-IFN- α 180 μ g weekly plus RBV 800 mg daily for either 12 weeks or 24 weeks. They showed significant correlation of plasma trough concentration of RBV with SVR on day 29 and week 12 of treatment. An SVR of 84% was achieved when plasma concentration was >2 mg/L while only 66% SVR was achieved when plasma concentration was below 2 mg/L at day 29 of combination treatment

($P=0.002$). Ribavirin plasma trough concentration was found to predict SVR independently at day 29 of combination treatment using multivariate analysis. Higher levels achieved higher rates of SVR. This study has shown the need to maintain adequate levels to achieve good SVR rates even in genotype 2 and 3 HCV infections unlike previously when TDM was advised mostly for genotype 1 and 4 infections.

Muñoz-de-Rueda et al⁶⁴ showed in 119 patients that though high plasma concentrations of RBV improved rates of SVR in HCV genotype 1 patients, their study did not favor TDM to optimize regular treatment. They showed that attaining $C_{\text{trough}} > 5 \text{ mg/L}$ improves rates of SVR and only such high concentrations correlated with SVR at W4, 12, 24, 48, and 72. At routine therapeutic doses administered, attaining such concentrations in patients based on body weight rarely happens. Even if such concentrations can be attained by measuring RBV and subsequently increasing dose, maintaining such high levels would only result in significant dose-dependent side effects warranting dose reduction or discontinuation compromising SVR. In spite of the findings of this recent study, TDM may be beneficial as evidenced by several other studies mentioned above. The reason for heterogeneity in results across studies is possibly multifactorial (differing clinical profile of patients, ethnicity, genetic influences on drug metabolism, sampling differences, comorbidities, and co-medications). Prospective randomized studies showing definite benefit in employing TDM of RBV, defining suitable therapeutic ranges to be maintained is needed before recommending regular use of TDM for all HCV patients on RBV.

Correlation of Plasma Concentration with Anemia: Evidence for and Against it

Ribavirin-induced hemolytic anemia is the most significant side effect which can affect achievement of SVR and favorable outcome by virtue of anemia-related dose reduction/treatment discontinuation. Adequate exposure of RBV in this regard for the full duration of treatment is necessary for clearing HCV from the body. Higher concentrations of RBV need to be achieved in genotype 1 patients to achieve SVR; thereby, these patients frequently experience hemolytic anemia. To strike a balance between efficacy and toxicity is a challenge. Lindahl et al⁶⁵ showed that decline in Hb levels associated with RBV has no correlation with dose per kilogram body weight but with plasma RBV concentration. Maeda et al⁶⁶ reported a drop in Hb levels below 8.5 g/dL when steady-state plasma concentration was $> 3500 \text{ ng/mL}$, and suggested RBV be dosed based on renal function as clearance of RBV was found to be dependent on creatinine clearance. A similar concentration threshold ($> 3.5 \text{ } \mu\text{g/mL}$) was reported by Arase et al⁵⁹ above which the incidence of RBV-induced anemia was

high, requiring EPO/blood transfusion. Saito et al⁶³ in a study of 97 Japanese patients showed significant correlation between drop in Hb levels and RBV serum concentrations during the first week of combination treatment. A relationship was also shown between RBV concentration on day 14 and day 28 and drop in Hb. However, maximum drop was seen in the first week supporting the idea of early monitoring of RBV to predict and limit treatment-related side effects.

Uchida et al⁶⁷ showed correlation between early RBV concentrations and fall in Hb (during the first week) and found concentrations to be higher during the first week in those patients who had discontinued treatment due to anemia. Donnerer et al⁶⁸ however, reported that steady-state serum concentrations of RBV (trough levels at week 12) did not predict drop in Hb levels. Inoue et al⁶⁹ also did not find a correlation between plasma RBV concentration and fall in Hb. However, they showed significant correlation between erythrocyte RBV concentration and fall in Hb. Most studies apart from few studies have shown good correlation between RBV plasma/serum concentration and anemia. Steady-state levels $> 3.5 \text{ } \mu\text{g/mL}$ are associated with high levels of Hb drop ($> 8.5 \text{ g/dL}$). Hence, steady-state levels of RBV should be preferably not exceed $3.5 \text{ } \mu\text{g/mL}$. Levels exceeding this threshold would jeopardize successful continuation of treatment and thus affect achievement of SVR.

Since, the fall in Hb starts on early in treatment, peaks within 2–4 weeks of treatment, early monitoring would be beneficial in this aspect to predict and prevent RBV-induced anemia. As steady-state concentrations of RBV are attained only after 4 weeks, it is inaccurate to interpret concentrations measured before this time as this may not be representative of true concentrations. However, a recent study⁵⁸ has shown relationship between early RBV concentration and steady-state concentration, thus allowing prediction of steady-state concentrations based on early levels measured. This can help in predicting drop in Hb early on in the treatment and thus early monitoring may be indeed beneficial. This is especially beneficial in those with renal dysfunction, HIV co-infection, co-medications likely to worsen anemia.

Utility of Therapeutic Drug Monitoring in Special Clinical Situations

Ribavirin Monitoring in Renal Impairment

Ribavirin kinetics is dependent on renal function. Monitoring RBV to individualize dosage in end-stage renal disease (ESRD) patients may be useful as they have frequent side effects and to attain levels which can achieve good SVR and acceptable toxicity is difficult as use of conventional doses of RBV is not possible on account of accentuation of Hb fall in the already anemic ESRD patients. Moreover, dialysis does not efficiently remove RBV.⁷⁰

Gupta et al⁷¹ showed that roughly 2.6% of RBV is removed after a 4-hour hemodialysis (HD) session (with 400 mg per day dosing). Earlier RBV and IFN combination therapy was not being used in HD patients with HCV infection. Rendina et al⁷² for the first time demonstrated in a large number of patients that combination therapy of PEG-IFN- α and low-dose RBV can be used to successfully treat CHC patients who are on hemodialysis with RBV dose adjustment based on plasma concentrations and Hb levels. Deltenre et al⁷³ recently showed that an adapted regimen of RBV starting with 600 mg per week and EPO as soon as Hb falls below 10 g/dL was effective in patients with ESRD (on hemodialysis) who were candidates for renal transplantation. Increasing the dose of EPO from the very start of treatment was quite effective in preventing anemia in such patients without reducing RBV dose. SVR achieved (40% in genotypes 1 and 4; 86% in genotypes 2 and 3) with such a preventive strategy was comparable with rates achieved in normal patients. But the study was inconclusive as to the superiority of adaptive or preventive strategy. The optimal dose range required in such patients still needs to be determined.

Van Leusen et al⁷⁴ have attempted to define a therapeutic range (RBV dose of 200 mg every other day) to achieve good SVR and reduce side effects in hemodialyzed patients with CHC infection. The suggested trough level of 1.5–2.5 $\mu\text{g/mL}$ achieved an SVR of 60% in HCV genotype 1/4 and 100% in genotype 2/3 patients. The problem of small sample size (7 patients) and the need for red cell transfusions in more than half the patients in spite of weekly increase in EPO dose still leave the question of optimal trough level in such patients unanswered. Carriero et al⁷⁵ demonstrated poor tolerability of 200 mg daily RBV (fixed dose) in hemodialyzed CHC patients with a 71.4% dropout rate and less than one-third patients attaining SVR.

Hence, TDM in CHC patients undergoing dialysis to tailor RBV dose is useful to improve outcome as well as minimize side effects, considering the deleterious effect HCV has on transplantation (like allograft nephropathy and diabetes), hence the need to eradicate HCV prior to transplantation.⁷⁶ The CHC infection increases morbidity and mortality in transplant recipients.⁷⁷ Combination treatment of RBV and PEG-IFN- α is usually contraindicated in patients who have already undergone renal transplantation due to the risk of allograft rejection.⁷⁸ Close monitoring of RBV with careful adjustment of dose based on levels and treatment response with judicious use of EPO to limit hematological side effects under expert supervision seems to be appropriate for patients with CHC infection who are candidates for renal transplantation to achieve SVR and thus improve clinical outcome.

Postliver Transplantation

Hepatitis C virus-related liver disease is the commonest indication for LT in the Western countries. Relapse of

HCV infection following LT (for HCV-related cirrhosis) is almost universal. Persistent HCV infection results in significant graft-related damage (more so in genotype 1b; cirrhosis, submassive necrosis, and fibrosing cholestatic hepatitis) and poor clinical outcome.^{79,80} Combination treatment of RBV plus PEG-IFN- α has been shown to be beneficial in the treatment of recurrent HCV infection post-LT. A high frequency (40–50%) of dose reduction/discontinuation of treatment in post-LT CHC patients has been reported on account of hemolytic anemia.⁷⁴

Ribavirin-induced hemolysis is more pronounced on account of higher prevalence of anemia and renal dysfunction in such patients. Concomitant immunosuppressive drugs (calcineurin inhibitors) also worsen RBV-induced hemolysis by causing nephrotoxicity leading to reduced levels of EPO. Jain et al⁸¹ assessed the role of RBV C_{trough} for relevance in the management of post-LT patients (on tacrolimus) with respect to toxicity, efficacy, renal dysfunction, and drug interactions. The study surprisingly found similar concentrations in plasma and whole blood with wide variation in concentrations achieved (the reason was not apparent in the study though the potential of tacrolimus to selectively bind to red cells and inhibit RBV binding was speculated). Higher concentrations were seen in patients with renal dysfunction (creatinine clearance <70 mL/min) who were on low-dose RBV. Patients with co-infection on protease inhibitors had significant drug interactions on account of inhibition of cytochrome p450 enzymes resulting in high RBV concentrations contributing to side effects. Eleven of 22 patients required EPO to counter-fall in Hb. Seven patients who cleared the virus had lower RBV concentrations while the remaining 9 patients who did not clear the virus had higher concentrations. There was no clear correlation between RBV concentration and the need for EPO as well as treatment response.

Dumortier et al⁸² in post-LT patients with CHC infection compared plasma concentrations of RBV in the LT group with nontransplanted control cohort. The dose used in LT group was lower than in the control group but the plasma concentration was similar in both groups, which was speculated to be on account of renal impairment in the LT group. A higher decline in Hb was seen in the LT group while similar treatment response was observed in both the groups. Though, the study supported RBV plasma concentration measurement to optimize treatment in LT patients, at present there is insufficient evidence to prove the benefit of TDM like reduction of treatment discontinuation/improvement in SVR. More studies are needed in LT patients to demonstrate the definite benefit of TDM in such patients. However, the presence of concomitant renal dysfunction and co-medications with interactions warrant monitoring as such complex clinical situations cannot be managed with blind dose reductions or standard protocols. Hence, treatment should be individualized based on measured levels.

Human Immunodeficiency Virus–Hepatitis C Virus Co-infection

Patients with HIV-HCV co-infection tend to have a more severe course and unfavorable clinical outcome. Lesser rates of SVR have been reported in co-infected patients.⁸³ Standard of care is the combination therapy of PEG-IFN plus lower dose RBV. Though increasing the dose of RBV improves chances of attainment of SVR, hematological side effects potentially affect achievement of SVR by virtue of dose reductions. Co-medications with significant drug interactions complicate the treatment of HCV infection. Crespo et al⁸⁴ in 94 HCV-HIV co-infected HCV treatment-naïve patients who received RBV and IFN combination therapy showed poor correlation between RBV serum concentration and Hb drop. They found significantly higher drops in those who received zidovudine. Serum concentration of RBV at weeks 4 and 12 was found to be lower in HCV genotype 1 and 4 infected patients who achieved rapid virological response (RVR) and this parameter was poor at identifying responders and nonresponders. A potential drug interaction between tenofovir and RBV, wherein RBV levels increased with concurrent administration of tenofovir, was addressed and speculated that tenofovir metabolites which accumulate in the RBC could have interfered with the degradation of RBV. The authors concluded that early monitoring of serum concentration of RBV is of little utility on account of poor correlation of early serum levels with either toxicity or efficacy. However, Rendón et al⁸⁵ in a similar study on 98 HIV-HCV co-infected individuals on combination therapy showed correlation between early plasma levels and drop in Hb levels which was predictably higher in those who received zidovudine. Higher plasma levels of RBV at week 4 (mean level 2.71 µg/mL) was associated with mean Hb drop of 2.5 g/dL in such patients, mean Hb drop in those receiving zidovudine being 2.7 g/dL. Higher RBV levels were seen in those who achieved EVR. No significant interaction with tenofovir or other antiretroviral drugs was found. All patients had normal glomerular filtration rate (GFR). Genotype 3 and plasma levels of RBV at week 4 independently predicted EVR. Their data supported early monitoring of plasma RBV levels to predict efficacy and toxicity and optimize treatment.

Aguilar Marucco et al⁸⁶ demonstrated a similar correlation in genotype 1 and 4 co-infected patients between plasma RBV C_{trough} and EVR/SVR, the cutoff being 1.6 µg/mL. A large decline in Hb (>4 g/dL) with C_{trough} >2.3 µg/mL was seen. Nicot et al⁸⁷ studied the correlation of serum levels of RBV and PEG-IFN- α and viral response at weeks 12 and 24 in patients with co-infection. No significant change in IFN levels was seen between responders and nonresponders. However, higher RBV levels were reported (2.32 µg/mL) in responders than in nonresponders (1.83 µg/mL), particularly in those infected with HCV 1

and 4 genotypes (2.672 µg/mL). Dahari et al⁸⁸ reported low RBV plasma concentrations during the first 14 days of treatment, probably reflecting extensive accumulation of RBV and its metabolites in the RBCs. The authors demonstrated significantly higher levels of RBV attained from the 2nd to 8th week in those who achieved SVR ($P < 0.02$). A higher decline of Hb was noted in those who achieved SVR during the first month of therapy (3.9 g/dL for SVR and 1.4 g/dL for nonresponders).

Piedoux et al⁸⁹ have demonstrated how TDM of RBV based on achieving cutoffs at week 12 in HCV patients even with co-infection can produce similar rates of SVR as in mono-infected patients. Three groups of patients comprising mixed population of HCV mono-infected and co-infected patients, namely, A1 (those who achieved RBV plasma level cutoffs at week 12), A2 (those who did not), and B1 (RBV concentration not measured) were studied to determine the relative impact these groups had on efficacy/SVR. It was found that the achievement of SVR was strongly predicted by the achievement of cutoff RBV levels and was independent of co-infection. In the presence of co-infection, achieving cutoffs produced similar rates of SVR as those without. Development of hematological side effects requiring dose reductions may hamper the achievement of such cutoff levels. The optimal therapeutic levels to be maintained still needs to be determined in such patients though it has been proposed that levels should not exceed 2.5 µg/mL¹⁴ at steady-state to avoid side effect-related dose reductions. Barring one study, data from other studies support the monitoring of RBV plasma concentration to achieve sufficient exposure of RBV to clear the virus, at the same time limit side effects. The HIV co-medications responsible for significant interactions support the use of TDM.

Feasibility of Therapeutic Drug Monitoring in Resource-limited Settings

Monitoring of RBV cannot be routinely carried out in resource-limited settings. Facilities for TDM are limited in developing countries including India. With few centers offering facilities and expertise in clinical pharmacology, it may not be practical or feasible to advise monitoring for all HCV patients in India. Financial constraints, poor compliance, and less opportunity for regular follow-up limit the use of TDM. However, where facilities are available, TDM should be considered to improve SVR and limit toxicity. Those with renal impairment, HIV-HCV co-infection, co-medications (with significant drug interactions), and post-LT patients may particularly benefit from such monitoring.

Summary

Several new anti-HCV drugs targeting specific steps in the replication of the HCV have been developed in the recent

Figure 3 Hepatitis C virus genome has an open reading frame of 9600 nucleotide bases and codes for structural and nonstructural proteins. Nonstructural proteins such as NS3/NS4A protease, NS5B polymerase/NS5A are specific targets based on which several inhibitors have been developed and are in various stages of clinical development. These agents form part of STAT-C and hold promise for future therapy with IFN and RBV. These agents when used alone develop resistance rapidly and hence are used in combination treatment with approved agents and have shown promising results.

IFN: interferon; RBV: ribavirin; RNA: ribonucleic acid; STAT-C: specifically targeted anti-viral therapy for hepatitis C.

past and are in advanced phases of clinical development (broadly referred to as specifically targeted therapy for hepatitis C [STAT-C]). Protease inhibitors (NS3/4A) such as telaprevir and boceprevir though have shown promising data in phase 3 trials⁹⁰ are effective mostly in combination with existing therapy (Figure 3). Monotherapy with protease inhibitors leads to rapid emergence of resistance abrogating the benefit of these agents with higher rates of relapse. Though tolerability of these drugs is better than other agents, they are not devoid of them. To develop regimens free of either IFN or RBV with shorter duration of treatment is a challenge which presently has not been met. Ribavirin remains to be an essential agent in combination therapy to achieve SVR and prevent relapse. In particular, adequate exposure of RBV for complete duration of treatment is vital in this regard, as evidenced by poorer rates of SVR in patients who discontinued treatment on account of severe RBV-induced anemia/other side effects.

Higher concentrations of RBV are needed to achieve SVR in HIV-HCV co-infected patients, at the cost of development of severe anemia. To address the issues of both efficacy and toxicity, a concentration that limits side effects without the compromise of achievement of SVR is critical. In this regard, TDM has a definite role to play to optimize treatment. Though a definite relationship has been shown in several studies between RBV concentrations and SVR/anemia (Table 1), some studies in the recent past have discouraged routine use of TDM in all HCV patients on combination treatment. In this regard, randomized studies with rigorous methodology showing definite improvement of clinical outcome, significant reduction of treatment discontinuation/dose reductions, are needed before TDM of RBV can be employed for all HCV patients, irrespective of HCV genotype/clinical profile/comorbidities. However, where possible, TDM should be considered (depending on the availability of resources/facilities, possibility of regular follow-up, and compliance)

Table 1 Summary of studies showing relationship between ribavirin levels and anemia/sustained virological response in chronic hepatitis C patients on combination treatment. It includes studies involving patients with human immunodeficiency virus-hepatitis C virus co-infection and renal dysfunction.

Studies	Correlation of RBV levels with anemia	Correlation of RBV levels with SVR	HIV-HCV co-infection	Renal dysfunction
Lindahl et al ⁶¹ Genotype 1 and 2/3	Yes, nonlinear correlation	–	No	No
Maeda et al ⁶⁶	Yes, $C_{\text{trough}} > 3500$ ng/mL associated with Hb < 8.5 g/dL	–	No	No
Arase et al ⁵⁹ Genotype 1b	Yes, $C_{\text{trough}} > 3.5$ μ g/mL associated with large decline in Hb levels	Yes, $C_{\text{trough}} > 3$ μ g/mL at week 8 associated with higher SVR rates	No	No
Uchida et al ⁶⁷ Genotype 1 and 2	Yes, drop in Hb correlated with levels at days 14 and 28	–	No	No
Donnerer et al ⁶⁸	No correlation	–	No	No
Inoue et al ⁶⁹ Genotype 1 and 2	No correlation	–	No	No
Saito et al ⁶³ Genotype 1	Yes, correlation between ribavirin levels and Hb drop during the first week of treatment	No correlation	No	No
Lindahl et al ⁶¹ Genotype 1	Yes, levels > 3.66 μ g/mL associated with high incidence of anemia. Managed with EPO and blood transfusions.	Yes, 9 of 10 patients achieved SVR with levels > 3.66 μ g/mL.	No	No
Maynard et al ⁶² Genotype 1	–	Yes, week 4 levels > 2 mg/L associated with 67% SVR compared to 16% when levels < 2 mg/L	No	No
Loustaud-Ratti et al ⁵¹ Genotype 1	–	Yes, early global ribavirin exposure (AUC 0–12 hr and AUC 0–4 hr) correlated with SVR. Threshold of 1755 μ g/hr/L (AUC 0–4 hr) on day 0 of treatment achieved higher SVR	No	No
Van Leusen et al ⁷⁴ Genotype 1, 4, 2, and 3	–	Yes, 1.5–2.5 μ g/mL achieved SVR in 100% genotype 2 and 3 patients while only 60% SVR was seen in genotype 1 and 4 patients	No	Yes
Crespo et al ⁸⁴ Genotype 1	–	Poor correlation	Yes	No
Rendina et al ⁷² Genotypes 1 and none	Yes	Yes	No	Yes
Rendon et al ⁸⁵ Genotype 1, 2, 3, and 4	Yes, 2.71 μ g/mL (mean level)—Hb decline 2.5 g/dL	Yes	Yes	No
Deltenre et al ⁷³ Genotypes 1, 2, 3, and 4	Their data supported TDM to prevent toxicity by measurement of levels and use of EPO either by an adaptive strategy (increase EPO if levels fall below 10 g/dL) or preventive strategy	–	No	Yes
Aguilar Marucco et al ⁸⁶ Genotype 1 and 4	Yes, levels > 2.3 μ g/mL—Hb decline > 4 g/dL	Yes, cutoff level 1.6 μ g/mL	Yes	No
Pederson et al ⁵⁶ Genotype 2 and 3		Yes, day 29 and week 12 levels correlated with SVR, week 4 levels > 2 mg/L achieved 84% SVR	No	No
Muñoz-de-Rueda et al ⁶⁴ Genotype 1		Yes, however only high concentrations, that is, > 5 mg/L correlated with SVR at Weeks 4, 12, 24, 48, and 72	No	No

AUC: area under concentration curve; EPO: erythropoietin; Hb: hemoglobin; HIV-HCV: human immunodeficiency virus-hepatitis C virus; RBV: ribavirin; SVR: sustained virological response; TDM: therapeutic drug monitoring.

as dose adjustments made purely on the basis of body weight do not achieve desirable concentrations balancing efficacy and toxicity.

Ribavirin kinetics is dependent on renal function and hence dosing should be done accordingly. At least dosing done empirically based on body weight should be followed up by monitoring of levels once steady-state has achieved. Though steady-state is not achieved before 4 weeks, recent studies have shown the importance of early monitoring as Hb decline happens early in the treatment. Slavenburg et al⁵⁸ have shown correlation of early and steady-state concentration which paves way for the calculation of steady-state levels based on early measurements to intervene early on in the treatment, allowing necessary dose adjustments to improve tolerability and clinical outcome. More studies addressing this aspect with development of a kinetic formula predicting reliably steady-state concentrations based on early measurements (1st/2nd week) will be beneficial in the long run to optimize treatment. Hepatitis C virus patients with ESRD who are candidates for renal transplantation would benefit from individualized dosing based on levels achieved, as anemia is a significant problem in such patients on account of deficiency of EPO and RBV-induced hemolytic anemia aggravating the pre-existing anemia. Maintaining adequate levels to achieve SVR is also important prior to transplant to prevent allograft nephropathy/rejection. Judicious use of EPO/transfusions will combat this problem to an extent to prevent major dose reductions/discontinuation.

Human immunodeficiency virus-HCV co-infection, co-medications with significant interactions, post-LT patients would benefit from monitoring. Genotype 1 and 4 patients have poorer rates of achievement of SVR compared with genotype 2 and 3 patients. Such patients may also benefit from monitoring to achieve therapeutic concentrations (for complete duration of treatment) effecting SVR. Optimal therapeutic levels of RBV still need to be determined. The therapeutic range may vary depending on comorbidities/genotype. However, levels $>3.5 \mu\text{g/mL}$ at steady-state would result in significant anemia as shown in several studies and hence levels should preferably be maintained below this level. On the other hand, subtherapeutic levels would hamper SVR, and hence steady-state levels $>2 \mu\text{g/mL}$ would be beneficial in this regard. However, maintaining lesser levels would be prudent in those with co-infection/co-medications to prevent treatment discontinuation.

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CONFLICTS OF INTEREST

All authors have none to declare.

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