Ribavirin-Induced Anemia in Hepatitis C Virus Patients Undergoing Combination Therapy

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Abstract

The current standard of care for hepatitis C virus (HCV) infection – combination therapy with pegylated interferon and ribavirin – elicits sustained responses in only ~50% of the patients treated. No alternatives exist for patients who do not respond to combination therapy. Addition of ribavirin substantially improves response rates to interferon and lowers relapse rates following the cessation of therapy, suggesting that increasing ribavirin exposure may further improve treatment response. A key limitation, however, is the toxic side-effect of ribavirin, hemolytic anemia, which often necessitates a reduction of ribavirin dosage and compromises treatment response. Maximizing treatment response thus requires striking a balance between the antiviral and hemolytic activities of ribavirin. Current models of viral kinetics describe the enhancement of treatment response due to ribavirin. Ribavirin-induced anemia, however, remains poorly understood and precludes rational optimization of combination therapy. Here, we develop a new mathematical model of the population dynamics of erythrocytes that quantitatively describes ribavirin-induced anemia in HCV patients. Based on the assumption that ribavirin accumulation decreases erythrocyte lifespan in a dose-dependent manner, model predictions capture several independent experimental observations of the accumulation of ribavirin in erythrocytes and the resulting decline of hemoglobin in HCV patients undergoing combination therapy, estimate the reduced erythrocyte lifespan during therapy, and describe inter-patient variations in the severity of ribavirin-induced anemia. Further, model predictions estimate the threshold ribavirin exposure beyond which anemia becomes intolerable and suggest guidelines for the usage of growth hormones, such as erythropoietin, that stimulate erythrocyte production and avert the reduction of ribavirin dosage, thereby improving treatment response. Our model thus facilitates, in conjunction with models of viral kinetics, the rational identification of treatment protocols that maximize treatment response while curtailing side effects.

Introduction

130–170 million people worldwide are currently infected with hepatitis C virus (HCV) [1]. Over 70% of HCV infections become chronic and if untreated may lead to cirrhosis and hepatocellular carcinoma, necessitating liver transplantation [1]. The standard of care for HCV infection involves combination therapy with pegylated interferon and ribavirin [2]. Ribavirin alone does not elicit a lasting antiviral response [3–6], yet it substantially improves treatment response in combination with interferon [7–11]. For instance, whereas ~29% of the patients treated with interferon exhibited a sustained virological response (SVR), the response rate increased to ~56% upon addition of ribavirin [8]. Ribavirin, however, is associated with the side-effect, hemolytic anemia, which often renders therapy intolerable [4,12–15]. With the standard ribavirin dosage of 1000–1200 mg/day, 54% of the patients treated experienced a decline in the hemoglobin (Hb) level of over 5 g/dL, and 10% of the men and 7% of the women treated experienced an Hb decline of over 5 g/dL (normal Hb range: 14–16 g/dL) [15]. This drop in Hb often necessitates a reduction of ribavirin dosage, which significantly compromises treatment response [7,13,14,16]. The probability of achieving SVR is estimated to decrease from ~65% to ~45% when ribavirin dosage is reduced from ~15 mg/kg to ~7 mg/kg of body weight, in combination with pegylated interferon at 1.5 µg/kg of body weight [7]. Patients receiving fewer than 60% of the planned ribavirin doses had lower response rates [14], indicating that lower cumulative ribavirin exposure results in poorer treatment response [13,16]. The rates of relapse of infection following the end of treatment also increased upon lowering ribavirin dosage [14,16]. In a recent clinical trial where interferon was employed with telaprevir, a promising new inhibitor of HCV protease, response rates were lowest in patients who were not administered ribavirin [17], underscoring the importance of ribavirin in achieving SVR. Alternatives for patients who do not respond to combination therapy do not exist yet [2,18]. Significant efforts are underway therefore to identify treatment protocols that maximize response rates to combination therapy while curtailing side-effects [16,19–25]. A particularly promising strategy is to supplement combination therapy with growth hormones, such as erythropoietin, that stimulate erythropoiesis and thus avert the reduction of ribavirin dosage, potentially improving treatment response [26–31]. The predominant mechanism(s) of the anti-HCV activity of ribavirin remain to be established [32–34]. Mathematical models of viral kinetics have been developed that describe the antiviral activity of interferon and the enhancement of treatment response rates due to
Author Summary

The treatment of HCV infection poses a major global health-care challenge today. The current standard of care, combination therapy with interferon and ribavirin, works in only about half of the patients treated. Because no alternatives are available yet for patients in whom combination therapy fails, identifying ways to improve response to combination therapy is critical. Increasing exposure to ribavirin does improve response but is associated with the severe side-effect, anemia. One way to maximize treatment response therefore is to increase ribavirin exposure to levels just below where anemia becomes intolerable. A second way is to supplement combination therapy with growth hormones, such as erythropoietin, that increase the production of red blood cells (erythrocytes) and compensate for ribavirin-induced anemia. Rational optimization of combination therapy thus relies on a quantitative description of ribavirin-induced anemia, which is currently lacking. Here, we develop a model of the population dynamics of erythrocytes in individuals exposed to ribavirin that quantitatively describes ribavirin-induced anemia. Model predictions capture several independent observations of ribavirin-induced anemia in HCV patients undergoing combination therapy, estimate the threshold ribavirin exposure beyond which anemia becomes intolerable, suggest guidelines for the usage of growth hormones, and facilitate rational optimization of therapy.

ribavirin, and are being extended to predict the impact of new antiviral drugs [34–42]. Ribavirin-induced anemia, on the other hand, remains poorly understood [13,24,25,43–47] and precludes rational optimization of combination therapy.

Here, we construct a mathematical model of the population dynamics of erythrocytes that quantitatively describes ribavirin-induced anemia and informs future strategies for improving outcomes of combination therapy. Model predictions capture experimental observations of the accumulation of ribavirin in erythrocytes and the ensuing Hb decline in HCV patients following the onset of combination therapy, estimate the enhanced turnover rate of erythrocytes during therapy and the threshold ribavirin exposure beyond which anemia is intolerable, present guidelines for the optimal usage of growth hormone supplements, and provide a framework, in conjunction with models of viral kinetics, for rational optimization of combination therapy.

Results

Model formulation

Prior to the onset of treatment with ribavirin, the population of erythrocytes (RBCs) in an HCV infected individual is constant; a balance exists between RBC production and death (Fig. 1). Following the onset of treatment, ribavirin administered orally gets rapidly transported from the plasma to RBCs, where it is phosphorylated to its mono-, di- and tri-phosphate analogs (RMP, RDP, and RTP) [48]. Phosphorylated analogs are neither easily metabolized nor transported out of RBCs [48]. Consequently, ribavirin accumulates inside RBCs in the form of its phosphorylated analogs; the total intracellular concentration of ribavirin can be >100-fold its extracellular concentration [47]. This dramatic accumulation of ribavirin may induce oxidative damage and result in enhanced vascular death of RBCs [12]. Indeed, RBC lifespan decreased from 107±22 d in HCV patients not exposed to ribavirin to 39±13 d in HCV patients undergoing treatment with ribavirin [49,50]. The shortened RBC lifespan creates an imbalance between RBC production and death and results in a decline in the RBC population. Accordingly, Hb levels drop and patients become anemic. We construct a mathematical model to describe this dynamics of ribavirin-induced anemia (Methods).

Model predictions

Population dynamics of RBCs during treatment with ribavirin. We present model predictions in terms of the cumulative population, \( m(C,t) \), which is the population of RBCs at time \( t \) following the onset of treatment in which the concentration of ribavirin phosphorylated analogs, RXP, is less than or equal to \( C \) (Fig. 2A). \( \text{RXP comprises RMP, RDP, and RTP.} \) At the start of treatment \( (t=0) \), no cells contain RXP, and \( m(C,0) = N_0 \) for all \( C \), where \( N_0 \) is the steady population of RBCs prior to the onset of treatment. In other words, the population of cells carrying RXP at concentrations smaller than or equal to \( C \) is \( N_0 \) for all \( C \geq 0 \). At \( t=0 \), the production and death rates of RBCs are in balance (Fig. 2B), the hemoglobin level, \( Hb \approx H_{bi} \), and the average intracellular concentration of ribavirin, \( C_{avg} = 0 \) (Fig. 2C). With time, ribavirin accumulates inside cells. At any time \( t \geq 0 \), a distribution of RXP concentrations across cells emerges with cells exposed to ribavirin longer possessing higher concentrations of RXP. Thus, cells present from the start of treatment possess the highest concentration of RXP. At \( t=1 \) d, for instance, the latter cells possess RXP at the concentration \( C \approx 0.04C_{\text{max}} \), where \( C_{\text{max}} = k_pC_p^{\text{max}}/k_d \) is the maximum intracellular concentration of...
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An increase in the steady state plasma concentration of ribavirin, \(C^\text{\text{max}}_p\), which may be achieved with a higher dosage, results in an increase in \(C^\text{\text{avg}}_p\) and a decrease in \(Hb_{\text{avg}}\), illustrating the more severe anemia that results with increased ribavirin exposure. For instance, increasing \(C^\text{\text{max}}_p\) from 5 \(\mu\text{M}\) to 15 \(\mu\text{M}\) increases the total drop in \(Hb\), \(\Delta Hb = Hb_0 - Hb_{\text{avg}},\) from \(-2\) g/dL to \(-5\) g/dL (Fig. 3A). An increase in the intracellular phosphorylation rate, \(k_p\), (or a decrease in the loss rate of intracellular RXP, \(k_d\)) results in a higher \(C^\text{\text{avg}}_p\) for the same \(C^\text{\text{max}}_p\), which in turn elevates cell death rates and lowers \(Hb_{\text{avg}}\). Thus, \(\Delta Hb\) increases from \(-2.5\) g/dL when \(k_p = 50\) d\(^{-1}\) to \(-3.5\) g/dL when \(k_p = 80\) d\(^{-1}\) (Fig. 3B), illustrating that differences in the intracellular metabolism of ribavirin may contribute to inter-patient variations in the severity of ribavirin-induced anemia. Enhancing the RBC production rate, by increasing \(P_{\text{max}}\) (see Eq. (3)), (or lowering the sensitivity of the RBC death rate to ribavirin accumulation, by increasing \(C_0\) or decreasing \(\gamma\) (see Eq. (2))), reduces \(\Delta Hb\) (Fig. 3C). Administration of growth hormones, such as erythropoietin, enhances the RBC production rate, which reduces \(\Delta Hb\) for the same ribavirin exposure and thus improves the tolerability of ribavirin. Similarly, decrease in the intracellular inosine triphosphatase level, observed recently in some patients [51], may interfere with RTP activity and lower the sensitivity of the RBC death rate to ribavirin, which also reduces \(\Delta Hb\) and improves the tolerability of ribavirin.

Inter-patient variations. Inter-patient variability in the severity of anemia may arise from variations in the intracellular uptake, accumulation, or metabolism of ribavirin, as well as in the dependence of the RBC lifespan on ribavirin accumulation and in the sensitivity of the RBC production rate to changes in \(Hb\). To obtain a measure of this inter-patient variability, we calculated \(\Delta Hb\) using 500 different combinations of the values of the parameters, \(k_p, k_d, C_0\) and \(\gamma\), for a range of values of \(C^\text{\text{max}}_p\). The parameter values for each combination were chosen randomly from Gaussian distributions based on the mean values and confidence levels of the respective parameters obtained from comparisons of our model predictions with patient data (see below). Indeed, we find that inter-patient variations in \(\Delta Hb\) may be substantial for any ribavirin exposure (Fig. 4A) or intracellular accumulation (Fig. 4B). Further, the variation increases with increase in ribavirin exposure and intracellular accumulation.

Below, we compare our model predictions with experiments.

Comparisons of model predictions with patient data

We consider a recent study of the time-evolution of \(C_{\text{avg}}\) and \(Hb\) in 19 Japanese patients following the onset of combination therapy [47]. In this latter study, no reduction of ribavirin dosage is
We fit model predictions of $C_{avg}$ and $Hb$ to the data of the former 7 patients using $k_p$, $k_d$, $C_{50}$ and $\gamma$ as adjustable parameters. (Interferon may also induce anemia, but does so to a much smaller extent than ribavirin [13]. We therefore assume that the $Hb$ decline in patients undergoing combination therapy is primarily due to ribavirin.) We fix the remaining parameters based on previous studies or from analysis of independent experiments (Methods). Model predictions provide good fits to the data and yield estimates of $k_p$, $k_d$, $C_{50}$ and $\gamma$ (Fig. 5A). The fits suggest that our model is able to describe the underlying dynamics of ribavirin-induced anemia in HCV patients.

Interestingly, with the same parameter values, our model captures changes in $Hb$ and $C_{avg}$ from the other 12 Japanese patients, as well as an independent data set of $Hb$ decline in another group of HCV patients undergoing combination therapy [29] (Fig. 5B), validating our best-fit parameter estimates. Further, with the same parameter values, we estimate that the RBC lifespan is 38 days (95% CI: 19–55 days) in Japanese patients with $C_{avg} < 1000$ $\mu$M and 33 days (95% CI: 14–53 days) in Japanese patients with $C_{avg} > 1000$ $\mu$M. These estimates of the RBC lifespan are in close agreement with independent estimates, 39 ± 13 days, from measurements of alveolar carbon monoxide [49,50], presenting another successful test of our model. Finally, we find that our predictions of the dependence of $A\text{Hb}$ on $C_{\text{p}}^{\text{max}}$ and $C_{avg}$ using the same parameters above are also in agreement with observations in the Japanese patients [47] (Fig. 5C,D). Our model thus presents a robust description of ribavirin-induced anemia in HCV patients undergoing combination therapy.

Clinical implications

Our model has several clinical implications. First, it enables estimation of the threshold ribavirin exposure beyond which anemia is intolerable. Current treatment guidelines recommend a reduction of ribavirin dosage when $Hb$ decreases below 10 g/dL. We apply our model to predict $Hb_{avg}$ as a function of $C_{avg}^{\text{max}}$. We find that on average (when $Hb_0 = 14.4$ g/dL) $Hb_{avg} < 10$ g/dL when $C_{avg}^{\text{max}} > 13$ $\mu$M (Fig. 6A). Thus, steady state plasma concentrations above 13 $\mu$M would render ribavirin therapy intolerable. While the dependence of the peak plasma concentration on dosage following a single ribavirin dose has been determined [48], the dependence of $C_{avg}^{\text{max}}$ on dosage remains to be established. A description of the multiple dose pharmacokinetics of ribavirin, which also remains elusive [6,34,48,52,53], would establish the dosage corresponding to $C_{avg}^{\text{max}}$ of 13 $\mu$M that would render ribavirin intolerable.

Second, when $C_{avg}^{\text{max}}$ is above the threshold, our model allows estimation of the increase in RBC production, which may be achieved by administration of exogenous growth hormones such as recombinant erythropoietin, necessary to avert the currently recommended reduction of dosage. Because growth hormones also have side-effects [29,54], one strategy is to use them at levels just enough to increase $Hb_{avg}$ to 10–12 g/dL (rather than the pretreatment level), which renders ribavirin tolerable [16]. We apply our model to predict the level of RBC production necessary for achieving $Hb_{avg}$ of 10–12 g/dL for different values of $C_{avg}^{\text{max}}$ (Fig. 6B). Thus, when $C_{avg}^{\text{max}} = 15$ $\mu$M, RBC production rates of 8.44 and 10.2 million cells s$^{-1}$ are necessary for ensuring $Hb_{avg}$ of 10 and 12 g/dL, respectively. Increase in endogenous erythropoietin levels during therapy, also observed experimentally [43,55], results in an enhanced production rate of 8.1 million cells s$^{-1}$, which is 3.5-fold higher than the basal production rate (here 2.3 million cells s$^{-1}$ in the absence of ribavirin) but inadequate to achieve the desired $Hb_{avg}$. Hormone supplements may be employed to provide the balance of 0.34 or 2.1 million cells s$^{-1}$

reported. The patients were divided into two groups based on whether $C_{avg} < 1000$ $\mu$M (7 patients) or $C_{avg} > 1000$ $\mu$M (12 patients); the data are reported as the average within each group.

Figure 3. Factors influencing the dynamics of ribavirin-induced anemia. Changes in $Hb$ (red) and $C_{avg}$ (green) predicted by Eqs. (1)–(3) for different parameter values: A, $C_{\text{p}}^{\text{max}} = 5$ $\mu$M (solid lines), 10 $\mu$M (dashed lines) and 15 $\mu$M (dotted lines). B, $k_p = 50$ d$^{-1}$ (solid lines), 65 d$^{-1}$ (dashed lines), 80 d$^{-1}$ (dotted lines). C, $P_{\text{max}} = 5.4 \times 10^{12}$ cells d$^{-1}$ (solid lines), 8.3 $\times 10^{12}$ cells d$^{-1}$ (dashed lines), 1.1 $\times 10^{12}$ cells d$^{-1}$ (dotted lines). The other parameters are mentioned in Table 1. doi:10.1371/journal.pcbi.1001072.g003
increase in the RBC production rate to ensure $Hb_{avg}$ of 10 or 12 g/dL, respectively. This deficiency in RBC production that hormone supplements must compensate with ribavirin exposure (Fig. 6B).

**Discussion**

The ability to enhance treatment response rates renders ribavirin central to the treatment of HCV infection. Maximizing the benefit of ribavirin to patients requires striking the right balance between its antiviral activity and its treatment-limiting side-effect, hemolytic anemia. Rational approaches to therapy optimization thus rely on quantitative descriptions of both the antiviral and the hemolytic activities of ribavirin. Extant mathematical models predict the enhancement in treatment response due to ribavirin [34–42]. Ribavirin-induced anemia, however, remains poorly described and limits our ability to maximize treatment response. Here, we fill this gap by constructing a model of the population dynamics of RBCs that quantitatively describes ribavirin-induced anemia. By assuming that intracellular accumulation of ribavirin enhances RBC death rate in a dose-dependent manner, our model captures several independent observations of ribavirin-induced anemia in HCV patients undergoing combination therapy. In particular, our model predicts the dynamics of the accumulation of ribavirin in RBCs and the resulting decline of $Hb$ in patients following the onset of therapy, estimates the reduced lifespan of RBCs during therapy, and describes inter-patient variations in the severity of anemia, thus presenting a robust description of ribavirin-induced anemia, which, in conjunction with models of viral kinetics, may facilitate identification of treatment protocols that maximize the impact of ribavirin in the treatment of HCV infection.

Our model has clinical implications. First, it allows estimation of the threshold ribavirin exposure beyond which ribavirin-induced anemia becomes intolerable. For instance, with model parameters that describe ribavirin-induced anemia in the patients we considered (Fig. 5), we estimate that steady state plasma ribavirin concentrations above 13 µM would render ribavirin therapy intolerable. Determining dosage levels corresponding to this steady state plasma concentration requires knowledge of the pharmacokinetics of ribavirin, which is currently lacking [6,34,48,53]. Ribavirin pharmacokinetics is peculiar because of an unusually long elimination phase that follows rapid absorption and distribution phases upon oral dosing [48]. Standard absorption-elimination models of drug pharmacokinetics are unable to describe this long elimination phase. Models that include additional compartments have been proposed to capture the three-phase pharmacokinetics of ribavirin [52], but the biological origin of these compartments remains unclear. An additional complication is that the half-life of the elimination phase increases from 79 h following a single dose to 274–298 h following multiple dosing [48], suggesting that parameters that describe single dose pharmacokinetics may not apply to multiple dose pharmacokinetics. In the absence of rigorous models of ribavirin pharmacokinetics, one may have to rely on empirical relationships between the dosage and the resulting steady state plasma concentration following multiple dosing (e.g., [56]) to establish the dosage that would ensure tolerability of ribavirin while maximizing treatment response.

Second, our model suggests guidelines for the usage of hormone supplements, such as erythropoietin, which enhance RBC production and improve the tolerability of ribavirin. For instance, we predict that when ribavirin accumulates to a plasma concentration of 15 µM, the associated enhanced RBC death rate elicits a natural response that increases RBC production 3.5-fold, from 2.3 to 8.1 million cells s$^{-1}$. This response, however, is inadequate to suppress ribavirin-induced anemia adequately and renders ribavirin intolerable. We estimate then that growth hormone supplements must increase RBC production rate by an additional 0.34–2.1 million cells s$^{-1}$ to render ribavirin tolerable. This compensation that hormone supplements must provide increases with ribavirin accumulation. Identifying the dosage of
the growth hormones that induces the necessary RBC production requires knowledge of the dose-response relationships and of the pharmacokinetics of the growth hormones, which are yet to be fully elucidated [26–31].

Third, genetic variations that resulted in a deficiency in the enzyme inosine triphosphatase (ITPA) were recently found to protect HCV patients against ribavirin-induced anemia [51]. Deficiency in ITPA causes an increase in inosine triphosphate levels in RBCs, which is thought to interfere with RTP activity and thereby suppress the hemolytic potential of ribavirin. Because deficiency in ITPA is a clinically benign condition, therapeutic intervention to suppress ITPA presents a promising new strategy to curtail ribavirin-induced anemia without compromising the antiviral activity of ribavirin [51]. Our model may be adapted to inform the development of such an intervention strategy. In our model, the dependence of the death rate of RBCs on ribavirin accumulation, determined by Eq. (2) (Methods), would now be a function of the ITPA level. Thus, experiments that determine how variations in the ITPA level both in the absence and in the presence of ribavirin influence RBC lifespan would provide the

Figure 5. Comparisons of model predictions with experiments. A, Best-fits of model predictions (lines) of Hb (red) and Cavg (green) with experimental data (symbols) from 7 Japanese patients with Cavg<1000 µM [47]. We let Hbavg = 14.4 g/dL and Cmax = 7.5 µM following the mean reported values for these patients [47]. We use kp, kd, CS0, and γ as adjustable parameters. The remaining parameters are mentioned in Table 1. The resulting best-fit parameter estimates (95% CI) are: kp = 65 d−1 (47–84 d−1), kd = 0.5 d−1 (0.3–0.7 d−1), CS0 = 408 µM (189–628 µM), and γ = 10.2–1.8. Dashed lines show 95% confidence intervals on the predictions. B, Comparisons of our predictions (lines) of Hb (red) and Cavg (green) using the parameters above with data (symbols) from 7 Japanese patients with Cavg >1000 µM (solid circles) [47]. In the latter patients, the mean Hb = 15 g/dL and Cmax = 9.8 µM. We also show comparisons with independent data from 20 patients [29] (open circles) with mean Hb = 15.1 g/dL. Solid lines are predictions with Hb = 15.1 g/dL and Cmax = 9.8 µM, and the mean parameters mentioned above, and dashed lines represent standard deviations. C, D, Model predictions (lines) and experimental observations (symbols) of the reduction in Hb (ΔHb = Hbavg − Hbavg) under combination therapy as a function of C, Cmax, and D, Cavg, in 19 Japanese patients [47]. Solid lines represent predictions with the mean parameter values above and dashed lines represent standard deviations obtained from several hundred realizations of our model predictions for different combinations of the values of kp, kd, CS0, and γ generated randomly from distributions based on the best-fit parameter estimates and 95% confidence limits mentioned above.

doi:10.1371/journal.pcbi.1001072.g005
necessary inputs for our model to account explicitly for the role of ITPA in ribavirin-induced anemia. The resulting model would enable determination of the minimal inhibition of ITPA necessary to maintain ribavirin-induced anemia within tolerable limits. Conversely, using information of the ITPA level intrinsic to a patient, the model can be applied to predict the maximum ribavirin dosage that the patient can tolerate, thus presenting an avenue for personalizing the treatment of HCV infection.

**Methods**

**Model development**

We consider the RBC population in an individual at time $t$ following the onset of treatment with ribavirin ($t=0$) (Fig. 1). RBCs produced at different times in the interval from 0 to $t$ will have been exposed to ribavirin for different durations and accordingly have different intracellular levels of ribavirin. We define $n(C,t)\Delta C$ as the population of RBCs that contain ribavirin phosphorylated analogs, RXP, which comprises RMP, RDP, and RTP, at concentrations between $C$ and $C+\Delta C$ at time $t$. $n(C,t)$ is thus the number density of RBCs containing RXP at concentration $C$ at time $t$. The time evolution of $n(C,t)$ is governed by the following equation (Text S1)

$$\frac{\partial}{\partial t} n(C,t) = -\frac{\partial}{\partial C} \left[ Q(C,C_t)n(C,t) \right] - n(C,t) D(C)$$

(1)

The first term on the right-hand-side in Eq. (1) represents the change in $n(C,t)$ due to intracellular phosphorylation of ribavirin. $Q(C,C_t)=k_pC - k_dC$ is the net rate of increase of $C$ due to phosphorylation, $C_t$ is the intracellular concentration of (unphosphorylated) ribavirin, $k_p$ is the phosphorylation rate and $k_d$ is the rate of loss, including by possible slow dephosphorylation, of RXP. In vitro studies of ribavirin uptake by RBCs observe rapid (~10 min) equilibration of intracellular and extracellular ribavirin [53,57]. We assume therefore that $C_t(t) \approx C_t(0)$, the concentration of ribavirin in plasma. With twice daily oral administration of ribavirin, $C_t$ rises from zero at $t=0$ and reaches an asymptotic maximum, $C_t^{\text{max}}$, so that $C_t(t) = C_t^{\text{max}}(1-\exp(-1/t_0))$, where $t_0$ is the characteristic timescale of the accumulation of ribavirin in plasma [6,38].

The second term on the right-hand side of Eq. (1) accounts for the loss of RBCs due to their death. We assume that the death rate, $D$, of RBCs increases with $C$ as follows

$$D(C) = D_0 \left( 1 + \left( \frac{C}{C_0} \right)^\gamma \right)$$

(2)

where $D_0$ is the death rate of RBCs in the absence of ribavirin, $C_0$ is that value of $C$ at which the death rate doubles (or the lifespan halves) compared to that in the absence of ribavirin, and $\gamma$, analogous to the Hill coefficient, determines the sensitivity of $D$ to changes in $C$. (A saturable form for $D(C)$ appears inconsistent with available data; see Text S2, Fig. S1.)

Equation (1) is constrained by the initial condition that $C=0$ in all cells at the start of therapy, so that $n(C,0) = N_0 Q(C_t)$, where $N_0$ is the population of RBCs at $t=0$, and $Q(C)$ is the Dirac delta function, which satisfies $Q(C \neq 0) = 0$ and $\int Q(C) dC = 1$. In other words, the Dirac delta function ensures that no cells have RXP at non-zero concentrations at $t=0$. A second constraint on Eq. (1) is imposed by the boundary condition that when $t>0$, newborn cells contain no RXP so that $n(0,t) = P(t)/Q(0,C_t)$ (Text S1) where

$$P(t) = \frac{P_{\text{max}}^\theta t}{\theta^\theta + \left( \frac{N(t)}{V} \right)^\theta}$$

(3)

is the rate of production of RBCs at time $t$.

The production of RBCs by the bone marrow is regulated by a negative feedback mechanism involving the hormone erythropoietin [58]. Recent studies on modeling erythropoiesis elucidate the complexities involved in a quantitative description of this feedback mechanism [59–65]. Here, we employ Eq. (3) to capture the essential features of this negative feedback: As the population of RBCs, $N(t) = \int_0^\infty n(C,t) dC$, decreases, $P$ increases. $P_{\text{max}}$ is the maximum production rate of RBCs, which occurs when $N$ is
vanishingly small, $\theta$ is that value of the RBC population per unit volume of blood ($N/V$) at which $P = P_{\text{max}}/2$, $V$ is the volume of blood, and $b$, analogous to the Hill coefficient, determines the sensitivity of $P$ to changes in $N/V$. Eq. (3) provides good fits to independent measurements of the dynamics of the recovery of RBCs following phlebotomy (Text S3, Fig. S2).

Equations (1)–(3) present a model of the population dynamics of RBCs in individuals undergoing treatment with ribavirin. We solve the equations (see below) and obtain the population density, $n(C,t)$, and the corresponding cumulative population, $m(C,t) = \int_0^C n(C',t)dC'$, using which we predict the time-evolution of the hemoglobin level in blood, $Hb(t) = \frac{100v_c}{3V}N(t)$ where $v_c$ is the volume of a single erythrocyte; the average concentration of ribavirin in RBCs, $C_{\text{avg}}(t) = \frac{1}{N(t)} \int_0^C n(C,t)(C+C_i)dC$; and the average RBC lifespan, $1/D_{\text{avg}}$, where $D_{\text{avg}}(t) = \frac{1}{N(t)} \int_0^C n(C,t)D(C)dC$ is the average death rate of RBCs.

Solution of model equations using the method of characteristics

Equation (1) along with the initial and boundary conditions is equivalent to the following set of differential equations obtained using the method of characteristics (Text S4)

$$\frac{dS_i(t)}{dt} = -D(C_i)S_i(t)$$

$$S_i(t_i) = \begin{cases} N_0 & i = 0 \\ P(t_i)\Delta t & i = 1,2,3,... \end{cases}$$

$$\frac{dC_i(t)}{dt} = k_p C_i(t) - k_d C_i(t)$$

$$C_i(t_i) = 0$$

where $S_i$ is the subpopulation of cells born within an interval $\Delta t$ of $t_i = t\Delta t$ that survive at time $t$. $C_i$ is the concentration of RXP in the latter cells at time $t$. We solve Eq. (4) along with Eqs. (2) and (3) with $\Delta t = 0.01$ d using a program written in MATLAB (Text S5). We validate our solution methodology against an analytical solution that can be obtained in the limiting case when the RBC death rate is independent of RXP accumulation (Text S6, Fig. S3). We also ensure that $\Delta t = 0.01$ d allows accurate integration of Eq. (4) without compromising computational efficiency (Fig. S4). From the solution, we calculate the quantities of interest, viz., $m(C,t) = \sum_{n_i \leq C} S_i(t)$, $N(t) = \sum_{i} S_i(t)$, $C_{\text{avg}}(t) = C(t) + \frac{1}{N(t)} \sum_{i} C(t)S_i(t)$, and $D_{\text{avg}}(t)$

$$= \frac{1}{N(t)} \sum_{i} D(C_i)S_i(t).$$

Model parameters

We employ the following values of the model parameters unless stated otherwise. The average RBC lifespan in normal man is $\sim 120$ days [49,66], which corresponds to $D_0 = 0.0083$ d$^{-1}$. We let $b = 7$ following earlier studies [59] and obtain $P_{\text{max}} \approx 8 \times 10^{12}$ cells d$^{-1}$ from an independent analysis of blood loss experiments (Text S3). We fix $V = 5$ L and $v_c = 9 \times 10^{-14}$ L [67]. Using $Hb_0 = 14.4$ g/dL [47], we get $N_0 = 2.4 \times 10^{13}$ cells. We obtain $\theta$ from the initial steady state $P(0) = D_0N_0$. Further, we let $C_{\text{p}} = 7.5 \mu$M [47] and because ribavirin accumulates in plasma to its maximum concentration in $\sim 4$ weeks, we set $t_p = 5.4$ d [6,38]. The remaining parameter values $k_p = 65$ d$^{-1}$, $k_d = 0.5$ d$^{-1}$, $C_{\text{p}} = 408 \mu$M, and $\gamma = 1$ are obtained from best-fits of our model predictions to experimental data (Fig. 5A). We summarize model parameters and their values in Table 1.

**Fits of model predictions to patient data**

We fit model predictions to experimental data (Fig. 5A) using the nonlinear regression tool NLINFIT in MATLAB.

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**Table 1.** Summary of model parameters and their values employed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value (95% CI)*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$</td>
<td>Volume of blood</td>
<td>5 L</td>
<td>[67]</td>
</tr>
<tr>
<td>$v_c$</td>
<td>Volume of one RBC</td>
<td>9 $\times 10^{-14}$ L</td>
<td>[67]</td>
</tr>
<tr>
<td>$t_0$</td>
<td>Characteristic timescale of accumulation of RBV in plasma</td>
<td>5.4 d</td>
<td>[6,38]</td>
</tr>
<tr>
<td>$b$</td>
<td>Coefficient determining sensitivity of RBC production rate to changes in number per volume of RBCs</td>
<td>7</td>
<td>[59]</td>
</tr>
<tr>
<td>$D_0$</td>
<td>Death rate of RBCs in the absence of ribavirin</td>
<td>$8.3 \times 10^{-3}$ d$^{-1}$</td>
<td>[49,66]</td>
</tr>
<tr>
<td>$Hb_0$</td>
<td>Initial Hb level</td>
<td>14.4 g/dL</td>
<td>[47]</td>
</tr>
<tr>
<td>$C_{\text{p}}$</td>
<td>Steady state plasma RBV concentration</td>
<td>7.5 $\mu$M</td>
<td>[47]</td>
</tr>
<tr>
<td>$\theta$</td>
<td>RBC population per unit volume at which RBC production rate is half maximal</td>
<td>Determined from pretreatment steady state, $P(0) = D_0N_0$</td>
<td></td>
</tr>
<tr>
<td>$P_{\text{max}}$</td>
<td>Maximum production rate of RBCs</td>
<td>$8.4 \times 10^{12}$ cells d$^{-1}$</td>
<td>Best-fit (Text S3)</td>
</tr>
<tr>
<td>$k_p$</td>
<td>Phosphorylation rate of ribavirin in RBCs</td>
<td>65 (47–84) d$^{-1}$</td>
<td>Best-fit (Text S3)</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Loss rate of ribavirin phosphorylated analogs in RBCs</td>
<td>0.5 (0.3–0.7) d$^{-1}$</td>
<td>Best-fit (Fig. 5A)</td>
</tr>
<tr>
<td>$C_{\text{p}}$</td>
<td>Concentration of ribavirin phosphorylated analogs at which RBC death rate is twice the pretreatment value</td>
<td>408 (189–628) $\mu$M</td>
<td>Best-fit (Fig. 5A)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Coefficient determining sensitivity of RBC death rate to changes in concentration of ribavirin phosphorylated analogs</td>
<td>1 (0.2–1.8)</td>
<td>Best-fit (Fig. 5A)</td>
</tr>
</tbody>
</table>

*Typical values employed. Variations are indicated in the text and in figure legends. doi:10.1371/journal.pcbi.1001072.t001
Supporting Information

**Figure S1** Hemoglobin reduction as a function of the intracellular ribavirin concentration. Found at: doi:10.1371/journal.pcbi.1001072.s001 (0.09 MB PDF)

**Figure S2** Analysis of RBC recovery following phlebotomy. Found at: doi:10.1371/journal.pcbi.1001072.s002 (0.41 MB PDF)

**Figure S3** Validation of the solution methodology. Found at: doi:10.1371/journal.pcbi.1001072.s003 (0.39 MB PDF)

**Figure S4** Sensitivity of the numerical solution to the integration time step. Found at: doi:10.1371/journal.pcbi.1001072.s004 (0.37 MB PDF)

**Text S1** Derivation of equation (1) and its boundary condition. Found at: doi:10.1371/journal.pcbi.1001072.s005 (0.29 MB PDF)

**Text S2** Dependence of RBC death rate on intracellular ribavirin concentration. Found at: doi:10.1371/journal.pcbi.1001072.s006 (0.19 MB PDF)

**Text S3** Analysis of phlebotomy experiments.

**References**

33. [Author Contributions](#)

We thank Alan S. Perelson and Ruy M. Ribeiro for helpful comments.

**Author Contributions**

Conceived and designed the experiments: SMK NMD. Performed the experiments: SMK. Analyzed the data: SMK NMD. Contributed reagents/materials/analysis tools: SMK NMD. Wrote the paper: SMK NMD.

Found at: doi:10.1371/journal.pcbi.1001072.s007 (0.28 MB PDF)

**Text S4** Solution of model equations using the method of characteristics. Found at: doi:10.1371/journal.pcbi.1001072.s008 (0.30 MB PDF)

**Text S5** MATLAB program for solving model equations. Found at: doi:10.1371/journal.pcbi.1001072.s009 (0.19 MB PDF)

**Text S6** Validation of the solution methodology. Found at: doi:10.1371/journal.pcbi.1001072.s010 (0.20 MB PDF)
Ribavirin-Induced Anemia


