A formula to estimate the optimal dosage of ribavirin for the treatment of chronic hepatitis C: influence of ITPA polymorphisms

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Background: Greater cumulative exposure to ribavirin increases response to interferon–ribavirin combination therapy for hepatitis C but also induces more severe anaemia. Polymorphisms in the ITPA gene protect against ribavirin-induced anaemia. The maximum dosage of ribavirin that can be tolerated by patients with different ITPA polymorphisms remains unknown.

Methods: We developed a mathematical model of haemoglobin (Hb) decline in patients undergoing combination therapy. Using it to analyse published patient data, we estimated the average erythrocyte lifespan in patients with different ITPA polymorphisms. Coupled with a previous population pharmacokinetic study, we derived a formula for predicting the optimal ribavirin dosage, $D_{\text{opt}}$, above which anaemia becomes intolerable (Hb<10 g/dl).

Results: Our model provided good fits to patient data of ribavirin accumulation in erythrocytes and the ensuing Hb decline during therapy. With the current treatment protocol, the average erythrocyte lifespan was approximately 36 days in patients with wild-type ITPA activity, and approximately 43 days and 55 days, respectively, in patients with mild and moderate ITPA deficiency. Our model yielded a facile formula for estimating $D_{\text{opt}}$ given a patient’s weight, creatinine clearance, pretreatment Hb and ITPA polymorphism. Patients with moderate ITPA deficiency are predicted to tolerate twice the ribavirin dosage as patients with wild-type ITPA.

Conclusions: Our formula for $D_{\text{opt}}$ presents an avenue for personalizing ribavirin dosage. By keeping anaemia tolerable, the predicted optimal dosage may improve adherence, reduce the need for drug monitoring, and increase response rates. Response rates may be increased further by the higher dosages recommended for patients with ITPA deficiency.

Introduction

Worldwide, 130–170 million individuals are estimated to be living currently with HCV infection [1]. Combination therapy with pegylated interferon and ribavirin elicits a sustained virological response (SVR) in approximately 50% of the patients treated [2]. Addition of the recently approved protease inhibitors can increase SVR to approximately 70% [3,4], but triple therapy is associated with severe side effects and the possibility of the development of drug resistance [5]. Several studies suggest that response rates to combination therapy can be improved by greater cumulative exposure and/or adherence to ribavirin [6–8]. For instance, when the plasma ribavirin concentration was maintained by careful monitoring and dose adjustments at approximately 15 μM, significantly higher than the typical exposure level of approximately 8–10 μM, 9 out of the 10 patients treated were cured [9]. A key limitation in maintaining adequate ribavirin exposure, however, is its side effect, haemolytic anaemia [6,10]. Ribavirin accumulates within erythrocytes to concentrations up to 100-fold higher than in plasma [11] and decreases erythrocyte lifespan [12]; the average erythrocyte lifespan decreased from approximately 120 days in uninfected individuals to approximately 40 days in HCV-infected individuals undergoing combination therapy [13,14]. Correspondingly, the haemoglobin (Hb) level in the blood declines following exposure to ribavirin, often necessitating a reduction of ribavirin dosage and compromising treatment response [6–8,15,16]. Hormone supplements, such as recombinant human erythropoietin, can be used to compensate for ribavirin-induced anaemia but have their own side-effects [17,18]. Consequently, it would be useful clinically to be able to estimate the maximum ribavirin dosage that...
can be tolerated by a patient without requiring hormone supplements or treatment interruptions.

Recently, two functional variants in the inosine triphosphatase (ITPA) gene, present on chromosome 20, have been found to protect against ribavirin-induced anaemia [19]. When patients had a missense variant in exon 2 (P32T mutation in rs1127354) or a splice-altering polymorphism in intron 2 (rs7270101), or both, which resulted in a deficiency of the ITPA gene product, they experienced a significantly lower Hb decline after 4 weeks of ribavirin therapy than in patients with wild-type ITPA [20–22]. Patients with ITPA variants may thus be able to tolerate higher ribavirin dosages, potentially improving their chances of attaining SVR. The two functional variants occur at different frequencies in different ethnic groups [19]. Approximately 21%, 10% and 3% of Caucasian individuals exhibit mild, moderate and severe ITPA deficiency, respectively, whereas among East Asian individuals, the corresponding numbers are 0%, 27% and 3% [19]. Guidelines for tailoring the ribavirin dosage based on the ITPA polymorphisms are yet to be established.

Here, we constructed a mathematical model of ribavirin-induced anaemia that quantitatively described patient data and identified the maximum ribavirin dosage beyond which anaemia becomes intolerable. The model fit data of the accumulation of ribavirin in erythrocytes and the concomitant Hb decline in HCV patients with different ITPA polymorphisms undergoing combination therapy. Importantly, the model yielded a simple expression for the plasma ribavirin concentration at which Hb drops below 10 g/dl, rendering anaemia intolerable. The plasma ribavirin concentration has been shown to be a function of the ribavirin dosage, body weight and creatinine clearance [23]. Integrating our expression with the latter function, we derived a facile formula for estimating the maximum ribavirin dosage that can be tolerated by a patient with a given body weight, creatinine clearance, pretreatment haemoglobin level and ITPA polymorphism.

**Methods**

Mathematical model of ribavirin-induced anaemia

We constructed the Equations 1 and 2 to describe the population dynamics of erythrocytes in an HCV-infected individual undergoing combination therapy:

\[
\frac{dN}{dt} = P \cdot DN
\]

\[
\frac{dC_{\text{RXP}}}{dt} = k_p C_{PC} - k_d C_{\text{RXP}} - \frac{PC_{\text{RXP}}}{N}
\]

Here, the population of erythrocytes, \( N \), changes with time \( t \) following the onset of therapy at a rate determined by the difference between the rates of production of erythrocytes, \( P \), and their death, \( DN \). Erythrocyte production is regulated by a negative feedback involving the hormone erythropoietin [24], which we modelled using the Hill function, \( P = P_{\text{max}} \theta/([N/V]^b+\theta^b) \) [25], where \( V \) is the blood volume, \( P_{\text{max}} \) is the maximum production rate that occurs when the erythrocyte number density, \( N/V \), becomes vanishingly small, \( \theta \) is that value of \( N/V \) at which \( P = P_{\text{max}}/2 \) and \( b \) determines the steepness with which \( P \) rises as \( N/V \) decreases. During therapy, ribavirin administered orally reaches plasma and is rapidly transported into erythrocytes, where it gets phosphorylated into its mono-, di- and triphosphate analogues [26]. We denoted the latter analogues collectively by RXP and defined \( C_{\text{RXP}} \) to be the average concentration of RXP in the erythrocytes at any time, \( t \). Erythrocytes lack dephosphorylation enzymes and RXP cannot be transported out, resulting in a build-up of RXP within erythrocytes and a lowering of the erythrocyte lifespan [12,26]. Following a previous study [25], we wrote the erythrocyte death rate constant as \( D = D_0(1+C_{\text{RXP}}/C_{g0}) \), where \( D_0 \) is the death rate constant in the absence of ribavirin and \( C_{g0} \) is that value of \( C_{\text{RXP}} \) at which the death rate doubles or the lifespan halves. \( C_{\text{RXP}} \) increases as intracellular ribavirin, at concentration \( C_g \), gets phosphorylated with the rate constant \( k_d \) and decreases due to the loss of RXP which occurs with the rate constant \( k_f \). Further, because newly produced cells lack RXP, they lower the average concentration \( C_{\text{RXP}} \) additionally at the rate \( PC_{\text{RXP}}/N \) (Additional file 1). The latter term may be thought of as a dilution term, where the newly produced cells increase the cell population but not the total RXP and hence effectively lower the concentration of RXP in cells on average. Transport of ribavirin across the erythrocyte membrane is rapid [27,28], so that intracellular and plasma concentrations of ribavirin are in equilibrium; that is, \( C_g = C_{\text{RXP}} \), where \( C_g \) is the concentration of ribavirin in plasma. Finally, we recognized that \( C_{g0} \), on average, rises exponentially following the onset of therapy and reaches an asymptotic maximum, \( C_{\text{RXP max}} \), so that \( C_g = C_{\text{RXP max}} (1-\exp[-t/t_d]) \), where \( t_d \) is the characteristic timescale of the accumulation of ribavirin in plasma [29,30]. We ignored the oscillations in \( C_g \) between doses because these oscillations occur with a period (equal to the dosing interval, approximately 12 h) much smaller than the timescale of Hb decline (weeks) and thus are expected not to influence the latter dynamics significantly.

Equations 1 and 2 present a model of ribavirin-induced anaemia. We solved the equations with the initial conditions that at the start of therapy (\( t=0 \)) no RXP exists in erythrocytes (\( C_{\text{RXP}}=0 \)) and that the erythrocyte production and death rates are in balance (\( N[0]=N_0=D_0 \)). We employed the solution to predict...
the time-evolution of $\text{Hb}=(100/3)\nu_rN/V$ (where $\nu_r$ is the volume of one erythrocyte), the total average intracellular ribavirin concentration, $C_{avg}=C_t+C_{p\scriptscriptstyle{\text{RXP}}}$, and the average erythrocyte lifespan, $1/D_t$. 

Model parameters

The average erythrocyte lifespan in healthy individuals is approximately 120 days [14,31], so that $D_t=0.0083$ day. With $b=7$, following the literature [32], and $P_{max}=8.4\times10^{13}$ cells/day, we showed previously that our model described independent experiments of the dynamics of the recovery of the erythrocyte population following phlebotomy [25]. We determined $0$ by enforcing the initial condition $N_s=P/D=(3/100)V=\text{Hb}_0\nu_r$, where $V=5 l$, $\nu_r=9\times10^{-14}$ [33], and $\text{Hb}_0$ is the pretreatment Hb. We let $t_a=5.4$ days, following observations that the plasma concentration of ribavirin reaches an asymptotic maximum in approximately 4 weeks [30]. We set $\text{Hb}_0$ and $C_{p\scriptscriptstyle{\text{max}}}$ to values reported for different patient populations and obtained $k_p, k_d, C_{50}$ through fits to patient data. The parameter values are listed in Additional file 2.

Data, fitting and analysis

We first considered data of the time-evolution of $C_{avg}$ and Hb in 19 Japanese patients following the onset of combination therapy [11]. No reduction of ribavirin dosage was reported in this study. The patients were stratified into two groups depending on whether $C_{avg}<1,000 \mu M$ (7 patients) or $C_{avg}>1,000 \mu M$ (12 patients). The two groups had a mean $C_{p\scriptscriptstyle{\text{max}}}$ of 7.5 and 9.8 $\mu M$ and $\text{Hb}_0$ of 14.4 and 15.1 g/dl, respectively. We fit our model predictions of $C_{avg}$ and Hb to the mean data from the former patients and obtained estimates of $k_p, k_d$ and $C_{50}$. The data were digitized using Engauge Digitizer and the fitting performed using the nonlinear regression tool NLINFIT in MATLAB. The latter tool employs the Levenberg-Marquardt algorithm for optimization and, coupled with the subroutines NLPARCI and NLPREDICI, yields 95% CIs on the best-fit parameter estimates and predictions.

We evaluated the goodness of fit as follows. We first employed the Student’s $t$-test to examine whether the mean of the residuals was significantly different from zero. Next, we employed the Wald–Wolfowitz runs test to ensure that the residuals were mutually independent. Finally, we performed the Lilliefors test to ascertain that the residuals were normally distributed. We performed the tests using built-in functions in MATLAB.

To validate our model and parameter estimates, we then compared our model predictions with data from the latter patients above (in whom $C_{avg}>1,000 \mu M$) without any adjustable parameters. Using the best-fit estimates of $k_p, k_d$ and $C_{50}$ above, we obtained the mean evolution of $C_{avg}$ and Hb in the latter patients and using the subroutine NLPREDICI estimated 95% CIs.

We next considered data of Hb decline in approximately 300 Western patients stratified according to their ITPA polymorphisms [20]. The polymorphisms at the locus rs1127354 are C/C, A/C and A/A with the minor allele A, whereas at the locus rs7270101, the variants are A/A, A/C and C/C with the minor allele C. An ITPA deficiency variable was defined based on the predicted ITPA activity incorporating polymorphisms at both loci [20]. The patients were thus stratified into four groups: wild-type (with 100% ITPA activity), or with mild (60% ITPA activity), moderate (30% ITPA activity) and severe (<10% ITPA activity) ITPA deficiency. Data of Hb decline was reported for the first three groups. We fit our model to the mean data from each group with either $C_{50}$ or $k_d$ as an adjustable parameter. We ascertained the goodness of the fits following the procedure above.

Formula for optimal ribavirin dosage

Using the best-fit parameter estimates, we applied our model at steady state and obtained an expression for the dependence of the final steady state Hb level, $\text{Hb}_\infty$, on the plasma ribavirin exposure, $C_{p\scriptscriptstyle{\text{max}}}$. We combined this expression with the link between $C_{avg}$ and the dosage determined previously through a population pharmacokinetic study [23] and arrived at a formula for the optimal ribavirin dosage, $D_{50}$, beyond which anaemia became intolerable ($\text{Hb}_\infty<10 \text{ g/dl}$).

Results

Dynamics of ribavirin-induced anaemia

We present model predictions of the accumulation of ribavirin in erythrocytes and the ensuing Hb decline as a function of time following the start of therapy (Figure 1). Before the start of therapy, erythrocyte production and death rates were in balance: $\text{Hb}=\text{Hb}_0$ and $C_{avg}=0$. Gradually, $C_{avg}$ increased and resulted in enhanced erythrocyte death. The imbalance between production and death caused a decline in Hb. The resulting lower number density of erythrocytes induced an enhancement in the erythrocyte production rate. Eventually, erythrocyte production and death attained a new balance: Hb attained a new, lower steady state, $\text{Hb}_\infty$, and $C_{avg}$ asymptotically reached a plateau value $C_{p\scriptscriptstyle{\text{max}}}$.

Increasing the plasma ribavirin exposure, $C_{p\scriptscriptstyle{\text{max}}}$, resulted in greater accumulation of ribavirin within cells, and correspondingly greater net Hb decline, $\Delta \text{Hb}=\text{Hb}_0-\text{Hb}_\infty$. Thus, upon increasing $C_{p\scriptscriptstyle{\text{max}}}$ from 8 $\mu M$ to 12 $\mu M$, $C_{avg}$ increased from approximately 990 $\mu M$ to approximately 1,460 $\mu M$, and $\Delta \text{Hb}$ increased from approximately 3 g/dl to approximately 4 g/dl, indicating more severe anaemia (Figure 1).
Parameter estimation and model validation

We fit model predictions of $C_{avg}$ and Hb decline to the corresponding mean data from seven Japanese patients in whom $C_{pmax}$ was approximately 7.5 $\mu$M using $k_p$, $k_d$ and $C_{50}$ as adjustable parameters (Figure 2A). Our model provided good fits to the data. The residuals from the fits were mutually independent and normally distributed with means not significantly different from zero (Figure 2B). Further, with the resulting best-fit parameter values and without any adjustable parameters, our model captured data of $C_{avg}$ and Hb decline in another 12 Japanese patients in whom $C_{pmax}$ was approximately 10 $\mu$M (Figure 2C). The average erythrocyte lifespan estimated by our model at steady state was approximately 37 days and approximately 31 days in the two populations (Figures 2A and 2C), respectively, which agrees well with estimates of mean ± sd 39 ± 13 days from alveolar carbon monoxide measurements [14]. Our model thus quantitatively describes ribavirin-induced anaemia in HCV patients undergoing combination therapy.

The above data did not distinguish between patients with different ITPA polymorphisms. The resulting parameter estimates are thus indicative of mean population behaviour. We examined next the influence of ITPA polymorphisms on Hb decline.

Impact of ITPA polymorphisms

The precise mechanism underlying the increased tolerance of ribavirin as a consequence of ITPA deficiency remains unknown. Recently, ITP has been suggested to substitute for GTP in the synthesis of ATP via the activity of the enzyme adenylosuccinate synthase, thus averting the loss of ATP [34] and maintaining erythrocyte lifespan despite RXP accumulation. In our model, the lower susceptibility to ribavirin induced mortality implies higher $C_{50}$. We also considered the following alternative possibility: ITPA deficiency could compromise the dephosphorylation of ITP, which in turn might reduce the availability of phosphate donors and inhibit the phosphorylation of ribavirin, thereby lowering $k_p$. Accordingly, we fit our model to the data of Hb decline in patients with different ITPA polymorphisms undergoing combination therapy using either $C_{50}$ or $k_p$ as an adjustable parameter. We obtained similar fits in the two cases (Figure 3 and Additional file 3). The net Hb decline significantly decreased as the level of ITPA deficiency increased. Correspondingly, we estimated that the average lifespan of erythrocytes was approximately 36 days in patients with wild-type ITPA activity, and increased to approximately 43 days and approximately 55 days in patients with mild and moderate ITPA deficiency, respectively. These latter lifespans were independent of whether $C_{50}$ or $k_p$ was used as an adjustable parameter.
This increase in the erythrocyte lifespan contributed to the increased ribavirin tolerance with ITPA deficiency. We next applied our model to predict ribavirin dosages that would ensure that anaemia does not become intolerable in the different patient groups.

Optimal ribavirin exposure

Using the parameter values obtained above, we solved our model equations to determine $\text{Hb}_\infty$ as a function of $C_{p_{\text{max}}}$ for different values of $\text{Hb}_0$ (Figure 4). Current clinical guidelines indicate that $\text{Hb}<10 \text{ g/dl}$ renders anaemia intolerable [35]. We therefore identified the optimal plasma ribavirin exposure, $C_{\text{opt}}$, as that value of $C_{p_{\text{max}}}$ at which $\text{Hb}_\infty=10 \text{ g/dl}$. We found that for patients with wild-type ITPA activity $C_{\text{opt}}$ was approximately $7.5 \mu M$ when $\text{Hb}_0$ was approximately $12 \text{ g/dl}$ and rose to approximately $15.5 \mu M$ when $\text{Hb}_0$ was approximately $14 \text{ g/dl}$ (Figure 4A). In patients with ITPA deficiency $C_{\text{opt}}$ was higher, indicating greater tolerance. For instance, when $\text{Hb}_0$ was approximately $12 \text{ g/dl}$, $C_{\text{opt}}$ was approximately...

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**Figure 2. Parameter estimation and model validation**

(A) Best-fits of model predictions to the mean data from seven patients of the change in haemoglobin ($\text{Hb}$) and $C_{\text{avg}}$ under going combination therapy. In these patients, $C_{p_{\text{max}}}=7.5 \mu M$ and $\text{Hb}_0=14.4 \text{ g/dl}$. The best-fit parameters values (mean ± s.d) are $k_p=65±8$/day, $k_d=0.5±0.07$/day and $C_{50}=413±23 \mu M$. Dashed lines represent 95% CIs on the model predictions. (B) Residuals of the best-fits in (A) of $\text{Hb}$ decline and $C_{\text{avg}}$. The mean residuals are $0.032 \text{ g/dl}$ and $5.4 \mu M$, respectively, and are not significantly different from zero ($P=0.579$ and 0.541, respectively, using a two-tailed Student’s $t$-test). Further, the residuals are not significantly mutually dependent ($P=0.91$ and 1, respectively, using the runs test) and are not significantly deviant from being normally distributed ($P=0.98$ and 0.72, respectively, using the Lilliefors normality test). (C) Model predictions of $\text{Hb}$ decline and $C_{\text{avg}}$ compared to the mean data of 12 patients with $C_{p_{\text{max}}}=9.8 \mu M$ and $\text{Hb}_0=15.1 \text{ g/dl}$ using the above best-fit parameter values. Dashed lines represent 95% CIs. The remaining parameters are listed in Additional file 2.
10 μM and approximately 16 μM in patients with the mild and moderate ITPA deficiency, respectively (Figure 4B). These predictions of \( C_{\text{opt}} \) agreed well with an expression that we derived by analysing our model at steady state (Additional file 4) as shown in Equation 3:

\[
C_{\text{opt}} = f_{\text{ITPA}} \left( \frac{15.6}{10+61-Hb_0} \left( \frac{10}{Hb_0} \right)^2 + 7.7 \right) - 63 \] (3)

Figure 3. ITPA polymorphism and ribavirin-induced anaemia

Best-fits of model predictions to the mean data of the change in the haemoglobin (Hb) level during ribavirin therapy in patients with (A) wild-type ITPA activity, (B) mild ITPA deficiency (60% of the wild-type ITPA activity) and (C) moderate ITPA deficiency (30% of the wild-type ITPA activity). The fits were obtained with \( C_{\text{opt}} \) as the adjustable parameter. The best-fit values of \( C_{\text{opt}} \) (mean ± sd) are 526 ± 22 μM, 683 ± 14 μM and 1,080 ± 126 μM for the three datasets, respectively. Fits using \( k_p \) as the adjustable parameter are presented in Additional file 3. Parameter values employed are \( k_p = 65.3 \) day, \( k_d = 0.5 \) day and \( C_{p_{\max}} = 10 \) μM. The remaining parameter values are listed in Additional file 2 (the sensitivity of the fits and the resulting predictions to \( C_{p_{\max}} \) are examined in Additional file 8). The insets show residuals of the best-fits to the data. In (A) the fit was performed using data until day 84, because significant dose reduction was reported subsequently. Using all of the data points, however, did not alter the fits or parameter estimates significantly. The mean residuals are -0.026, -0.0013 and 0.0184 g/dl, respectively, and are not significantly different from zero (P=0.752, 0.972 and 0.905, respectively, using a two-tailed Student’s t-test). Further, the residuals are not significantly mutually dependent (P=1.0, 0.4 and 0.4, respectively, using the runs test) and are not significantly deviant from being normally distributed (P=0.1, 0.053 and 0.96, respectively, using the Lilliefors normality test).
Here, $Hb_0$ is in units of g/dl, and $f_{ITP}$ is a factor that accounts for ITPA variations: $f_{ITP}=1, 1.3, \text{ and } 2.05$ for patients with wild-type ITPA activity, and mild and moderate ITPA deficiency, respectively (Figure 4B).

**Optimal ribavirin dosage**

Using a population pharmacokinetic analysis, Bruchfeld et al. [23] have identified a link between the plasma ribavirin exposure, $C_{pmax}$, and the corresponding dosage: $D_{opt} = 0.244I_d(0.122CL_{cr}+0.0414BW)$, where $I_d$ is the dosing interval (h), $CL_{cr}$ is the creatinine clearance (ml/min), $BW$ is the body mass (kg), and $Dose$ is the dosage (mg). By letting $C_{pmax}=C_{opt}$ in the above expression, we obtained a formula (Equation 4) for the optimal ribavirin dosage, $D_{opt}$, at which $Hb=10$ g/dl:

$$D_{opt} = 0.244I_d(0.122CL_{cr} + 0.0414BW)$$

Accordingly, with $CL_{cr}=100$ ml/min, $BW=40$ kg and wild-type ITPA activity, $D_{opt} \approx 300$ mg twice daily for a patient with $Hb_0=12$ g/dl and $\approx 600$ mg twice daily with $Hb_0=14$ g/dl (Figure 5A). For a patient with a higher $BW=120$ kg, $D_{opt}=400$ mg twice daily if $Hb_0=12$ g/dl. Similarly, $D_{opt}=900$ mg twice daily with $BW=120$ kg and $Hb_0=14$ g/dl but a higher $CL_{cr}=120$ ml/min (Figure 5B). With mild and moderate ITPA deficiency, $D_{opt}$ is higher than the wild-type by a factor of 1.3 and 2.05, respectively (determined by $f_{ITP}$). Thus, when $BW=70$ kg, $Hb_0=13$ g/dl and $CL_{cr}=100$ ml/min, $D_{opt}=530$ mg twice daily with wild-type ITPA activity and $D_{opt}=690$ mg twice daily and 1,100 mg twice daily with mild and moderate ITPA deficiency, respectively (Figure 5C). Again, $D_{opt}$ increases with increasing $CL_{cr}$ for all ITPA polymorphisms (Figure 5D).

**Discussion**

The recent approval of two new HCV protease inhibitors as well as the rapid pace of the development of a large number of new direct-acting antiviral agents...
has raised hopes of a cure of HCV for all infected individuals [35,36]. Ribavirin appears indispensable to achieving this cure. For instance, in a clinical trial where the protease inhibitor telaprevir was administered with interferon, response rates were lowest in patients not administered ribavirin [37]. Adherence to ribavirin is compromised, however, by its side effect, haemolytic anaemia, which in turn lowers response. Significant efforts are underway, therefore, to identify optimal dosing protocols that would ensure adequate

Figure 5. Optimal ribavirin dosage

Model predictions obtained using Equation 4 of the optimal ribavirin dosage, \( D_{opt} \), as a function of (A) pretreatment Hb (Hb\(_0\)) and (B) creatinine clearance (CL\(_{cr}\)), for patients with wild-type ITPA activity with a body mass (BW) = 40 kg and BW = 120 kg. \( D_{opt} \) as a function of (C) Hb\(_0\) and (D) CL\(_{cr}\) for patients with wild-type ITPA activity, mild ITPA deficiency and moderate ITPA deficiency. Unless mentioned otherwise, the parameters were fixed at Hb\(_0\) = 14 g/dl, CL\(_{cr}\) = 100 ml/min, BW = 70 kg, \( k_p = 65.3/day \), and \( k_d = 0.5/day \). C\(_50\) was set to the best-fit values obtained in Figure 3, namely, 526 \( \mu \)M, 683 \( \mu \)M and 1,080 \( \mu \)M for patients with wild-type ITPA activity, mild ITPA deficiency and moderate ITPA deficiency, respectively. The remaining parameters are listed in Additional file 2.
exposure to ribavirin without rendering anaemia intolerable [8,25,38–42]. Several previous studies have emphasized the need for personalizing and  
regular monitoring of ribavirin exposure during the treatment of chronic hepatitis C [25,39,40,42,43]. Here, we have employed a mathematical model of ribavirin-induced anaemia that describes patient data of the accumulation of ribavirin in erythrocytes and the ensuing haemoglobin decline quantitatively and estimated the maximum plasma ribavirin exposure beyond which anaemia becomes intolerable. In conjunction with a previous population pharmacokinetic analysis [23], we have derived a formula that predicts the corresponding optimal ribavirin dosage. The formula allows personalizing the dosage of ribavirin based on body weight, creatinine clearance, pretreatment haemoglobin level and ITPA polymorphism.

Our model predicts that the ribavirin dosage that patients can tolerate can often be higher than the current weight-based guideline (400–700 mg twice daily) depending on the pretreatment haemoglobin and creatinine clearance. Further, the dosage can be significantly higher for patients with ITPA deficiency. Specifically, patients with moderate ITPA deficiency, who have about 30% of the wild-type ITPA activity, are estimated to tolerate dosages over twice that of patients with wild-type ITPA activity. The greater resulting exposure to ribavirin is likely to increase response rates. At the same time, our model predicts that the optimal dosage may also be smaller than the current guideline, in which case dosing based on the current guideline may lead to intolerable anaemia, necessitating dose reduction or the cessation of ribavirin administration. The optimal dosage predicted here would preclude intolerable anaemia, ensure greater adherence and therefore higher cumulative exposure to ribavirin and potentially improve treatment response.

While ITPA polymorphism is well-correlated with the reduced incidence of ribavirin-induced anemia [19–22], its impact on treatment response remains unclear. Some studies found no correlation between ITPA polymorphism and SVR [22,44], whereas others did observe a correlation [21,45]. Most studies examining the correlation between ITPA polymorphism and treatment response published so far are retrospective analyses of trials where ribavirin was administered following the current weight-based guideline. Whether response rates would increase significantly if higher dosages, as suggested by our present study, were administered to patients with ITPA deficiency remains to be ascertained.

In a previous study, we developed a more detailed model of ribavirin-induced anaemia that considered the accumulation of ribavirin phosphorylated analogues (RXP) within individual erythrocytes based on the duration of their exposure to ribavirin [25]. In our present model, the time-evolution of the ‘average’ concentration of RXP in the entire population of erythrocytes within an individual is considered. Analysis of patient data with the previous model yielded best-fits that suggested that the death rate of erythrocytes depends linearly on the intracellular concentration of RXP [25]. The present model can then be shown to be equivalent to the previous model so long as the variance in the accumulation of ribavirin across erythrocytes is small (Additional file 5). Indeed, for the first 6–8 weeks of therapy, most of the erythrocytes present in an individual are the ones that existed at the start of therapy (the pretreatment erythrocyte lifespan is approximately 120 days and gradually decreases to approximately 40 days during therapy). Ribavirin thus accumulates similarly in most cells for the first 6–8 weeks, rendering the variance in the concentration small (Additional file 6). Indeed, the fits of the present model to patient data of ribavirin-induced anaemia in the first 4–8 weeks following the start of therapy and the resulting best-fit parameter estimates were close to those obtained with our previous model. The advantage of the present model lies in its simplicity: whereas the previous model was based on a partial differential equation that was difficult to solve, the present model is based on two coupled ordinary differential equations, which are easily solved and also yield a simple formula that can potentially be employed at the bedside.

A mechanistic understanding of the ITPA deficiency-induced tolerance of ribavirin is currently lacking. We considered two plausible mechanisms. First, ITP could substitute for GTP in the synthesis of ATP, thus averting the loss of ATP and maintaining erythrocyte lifespan despite RXP accumulation [34]. We modelled this lower susceptibility to ribavirin-induced cellular mortality by a lower sensitivity of the erythrocyte lifespan to RXP, or a higher \( C_{50} \). Second, ITPA deficiency is expected to increase ITP levels in erythrocytes, which could limit the availability of phosphate donors and inhibit the phosphorylation of ribavirin. We modelled this as a reduced rate of phosphorylation of ribavirin, \( k_p \). Interestingly, with either \( k_p \) or \( C_{50} \) as an adjustable parameter, our model provided similar fits to patient data of Hb decline. The lifespan of erythrocytes estimated was also similar in the two cases. In the former case, it is conceivable that the higher ATP levels may increase phosphate donors and therefore allow greater phosphorylation of ribavirin and hence also increase \( k_p \). We therefore also obtained fits where both \( k_p \) and \( C_{50} \) were adjustable. Again, the fits were similar and did not influence the average lifespan or our predictions of optimal dosages (data not shown). Importantly, we found that whereas the average erythrocyte lifespan was approximately 36 days in patients with wild-type ITPA activity undergoing combination therapy, which

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is in agreement with independent measurements of approximately mean ±st 39 ±13 days using alveolar carbon monoxide measurements [14], the lifespan was approximately 43 days and approximately 55 days, respectively, in patients with mild and moderate ITPA deficiency. This increased lifespan corresponded to the increased tolerance of ribavirin in the latter patients. Yet, the erythrocyte lifespan was much lower in the latter patients than the normal lifespan of approximately 120 days in patients not exposed to ribavirin, indicating that mild and moderate ITPA deficiency confers only a partial relief from ribavirin-induced anaemia. Further, the optimal ribavirin dosages predicted were not sensitive to whether $k_p$ or $C_{50}$ was used as an adjustable parameter. Thus, our predictions hold regardless of which of these two mechanisms underlies ITPA deficiency-induced tolerance of ribavirin. Our model, however, does allow distinction between the two mechanisms. In the first mechanism, erythrocytes survive despite significant RXP accumulation, whereas in the second, RXP accumulation is compromised. Thus, measurement of the RXP accumulation within erythrocytes from patients with different ITPA polymorphisms would serve to delineate the underlying mechanism (Additional file 7). Associated gene expression patterns are now being explored [46], which may shed further light on these mechanisms.

Lindahl et al. [9] have reported that the ribavirin concentration achieved in plasma was somewhat smaller than that predicted by the population pharmacokinetic analysis [23]. Thus, the optimal dosage recommended by our formula is expected to be a conservative estimate of the maximum dosage beyond which anaemia becomes intolerable. A precise link between the plasma concentration and dosage would require a model of the multiple dose pharmacokinetics of ribavirin, which is currently lacking [26,28,47,48]. Further, we recognize that the optimal dosage is expected to be different across ethnic groups. For instance, Japanese patients are prescribed lower dosages than Western patients [49]. Thus, our prediction of the optimal dosage (Equation 4) is restricted to Western populations for which the correlation of Bruchfeld et al. [23] applies. In addition, factors other than those considered in the latter analysis (for example, see [50]) may lead to further inter-patient variations. By presenting a rationally identified target concentration and optimal dosage, our formula may minimize the need for subsequent monitoring of ribavirin exposure.

Finally, we note that our formula for the optimal ribavirin dosage (Equation 4) may be used even when ITPA polymorphisms cannot be easily determined, as in resource-constrained settings. Our formula assuming wild-type ITPA activity ($f_{ITP}=1$) would yield a conservative estimate of the dosage that can be tolerated given a patient’s body weight, creatinine clearance, and the pretreatment haemoglobin level.

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Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: Details of the derivation of model Equation 2 can be accessed via http://www.intmedpress.com/uploads/documents/AVT-12-OA-2501_Krishnan_Add_file1.pdf

Additional file 2: Typical values of model parameters employed can be accessed via http://www.intmedpress.com/uploads/documents/AVT-12-OA-2501_Krishnan_Add_file2.pdf


Additional file 4: Details of the derivation of the formula for optimal ribavirin exposure can be accessed via http://www.intmedpress.com/uploads/documents/AVT-12-OA-2501_Krishnan_Add_file4.pdf

Additional file 5: Details of the equivalence of model equations with the previous model of Krishnan and Dixit [25] can be accessed via http://www.intmedpress.com/uploads/documents/AVT-12-OA-2501_Krishnan_Add_file5.pdf


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References


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