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Review

The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus

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Abstract. Ribavirin, a broad spectrum antiviral agent, in conjunction with interferon forms the current standard of treatment for hepatitis C virus (HCV) infection in humans. While ribavirin alone fails to induce a significant antiviral response, in combination with interferon, ribavirin dramatically improves the long-term outcome of therapy. The predominant mechanism(s) of ribavirin action against HCV, are yet to be established. In this review, we examine the current status of our understanding of the metabolism, pharmacokinetics and mechanisms of the antiviral activity of ribavirin against HCV, all of which are central to the rational identification of improved treatment protocols.

Keywords. Mutagenesis, treatment response, viral dynamics, mathematical model, monotherapy, combination therapy.

Introduction

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a guanosine analog (Fig. 1) first synthesized in the 1970s [1], has been shown to exhibit antiviral activity against several RNA and DNA viruses both *in vitro* and *in vivo* [2–5]. Ribavirin was first approved for use in humans to treat respiratory syncytial virus infection in children. Today, ribavirin is used for the treatment of Lassa fever, respiratory syncytial virus and hepatitis C virus (HCV) infections of humans. Over 170 million individuals are estimated to be living currently with HCV infection [6]. In ~70% of the cases, HCV infection becomes chronic and if untreated may lead to cirrhosis, end-stage liver disease and hepatocellular carcinoma necessitating liver transplantation [7]. Treatment options for HCV infection are limited. In the 1980s, soon after the identification of HCV, interferon-α monotherapy was tried as a treatment for HCV infection but saw limited success [8]. In the 1990s, ribavirin was tested for activity against HCV [9–16]. While ribavirin monotherapy was unable to induce a significant antiviral response in HCV patients [9–13], in combination with interferon, ribavirin dra matically improved long-term response rates to therapy [14–16].

The long-term virological responses to anti-HCV therapy have been characterized as sustained virological response (SVR), end-of-treatment response (ETR) and non-response. SVR is defined as the loss of detectable plasma HCV RNA during treatment and its continued absence for at least 6 months after cessation of therapy [17]. ETR implies HCV RNA is undetectable when therapy is terminated. In this case, HCV RNA may subsequently rebound so that SVR is not attained. Non-response indicates detectable HCV RNA throughout the treatment period. In one study, which illustrates the dramatic impact of ribavirin addition, the fraction of patients treated who exhibited SVR increased from ~6% without to ~31%

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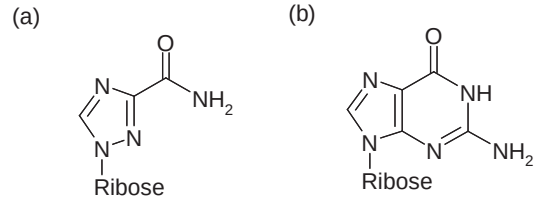

Figure 1. The chemical structures of ribavirin (*a*) and guanosine (*b*).

with ribavirin following 24 weeks of standard interferon therapy [14]. Following 48 weeks of interferon therapy, SVR increased from ~13% without to ~38% with ribavirin [14]. With pegylated interferon, which has better pharmacokinetic properties than standard interferon, response rates improved further [18–20]. For instance, following 48 weeks of therapy, SVR increased from ~29% without to ~56% with ribavirin [20].

No alternative therapies exist for nonresponders [21, 22]. Development of new anti-HCV drugs has been severely impeded by the lack of small animal models and efficient culture systems, and concern over the inherent ability of HCV to rapidly acquire antiviral drug resistance mutations [21, 22]. Consequently, combination therapy with pegylated interferon and ribavirin has become the current standard of treatment for HCV infection [18]. At the same time, significant efforts are under way to identify better treatment strategies that would improve long-term response rates [21–23]. The substantial improvements in treatment response rates induced by ribavirin suggest, promisingly, that tuning ribavirin therapy may improve response rates further. Maintenance therapy with ribavirin is already being assessed as an option for nonresponders [24].

Rational therapy optimization hinges on a fundamental understanding of the antiviral role of ribavirin. Remarkably, despite the widespread use of ribavirin over the past decade, the predominant mechanism(s) of action of ribavirin are yet to be established [9, 25, 26]. The pharmacokinetic profile of ribavirin has a long terminal elimination phase, which remains poorly understood [27]. The cause(s) of the synergy between interferon and ribavirin, which appears crucial in anti-HCV therapy [28], remain unclear. Notwithstanding, the promise of improving therapy for HCV infection has rekindled interest in ribavirin. Recent studies have made significant advances, unraveling various aspects of the effects of ribavirin on viral pathogenesis and treatment response. In this review, we examine the current status of our understanding of ribavirin and its role in the treatment of HCV infection.

Mechanisms of action

HCV is a hepatotropic flavivirus with a single linear positive strand RNA genome of ~10^4 nucleotides. The main target of HCV is the hepatocyte. The HCV lifecycle remains to be fully elucidated; most of the present understanding is from replicon systems and related viruses [see 29–31]. HCV replication proceeds entirely in the cytoplasm in association with cytoplasmic membranes. Following entry into hepatocytes, the positive strand RNA genome is translated into viral proteins that include the RNA-dependent RNA polymerase (RdRp) central to the replication process. RdRp employs the RNA genome as a template to form negative strand RNA intermediates, which in turn facilitate the formation of progeny positive strand RNA genomes. The latter genomes are packaged into new viral particles and released from infected hepatocytes into circulation.

HCV is a noncytopathic virus. Each infected hepatocyte may therefore give rise to a large number of progeny virions during its lifetime. Interferon is thought to act as an antiviral agent against HCV by inhibiting viral production from infected hepatocytes [17, 32]. It does this indirectly by inducing a large number of host cell genes that establish a non-specific antiviral state within an infected hepatocyte that may affect viral protein synthesis and viral RNA stability [17]. In addition, interferon may act against HCV via its effects on the immune system, viz., promotion of memory T-cell proliferation, natural killer (NK) cell activation, dendritic cell maturation and inhibition of T-cell apoptosis [17]. The result is a dramatic reduction in plasma HCV RNA following the onset of interferon therapy, which culminates in SVR in a small fraction of the patients treated [33].

Several mechanisms have been suggested for the broad spectrum antiviral activity of ribavirin, viz., direct inhibition of viral RNA replication, inhibition of the enzyme inosine-monophospate-dehydrogenase (IMPDH), immunomodulation, and mutagenesis [17, 25, 26, 34]. These mechanisms are illustrated in Fig. 2. Ribavirin has also been suggested to act synergistically with interferon [35, 36]. Experimental evidence suggests that different combinations of these mechanisms may be at play against different viruses. Here, we summarize available evidence for each of these possible mechanisms.

Direct inhibition of RNA replication

Ribavirin is phosphorylated inside cells to ribavirin mono-, di- and triphosphate (RMP, RDP and RTP, respectively) [37]. RTP, a guanosine triphosphate (GTP) analog, is incorporated into replicating RNA strands by viral RNA polymerases [37–40]. This erroneous incorporation may inhibit chain elongation and, in the extreme situation, cause chain termination. Crotty et al. [38] showed that ribavirin was incorporated in nascent poliovirus strands without causing chain termination and that ribavirin paired equally efficiently with both cytidine and uridine. However, cytidine and uridine incorporation

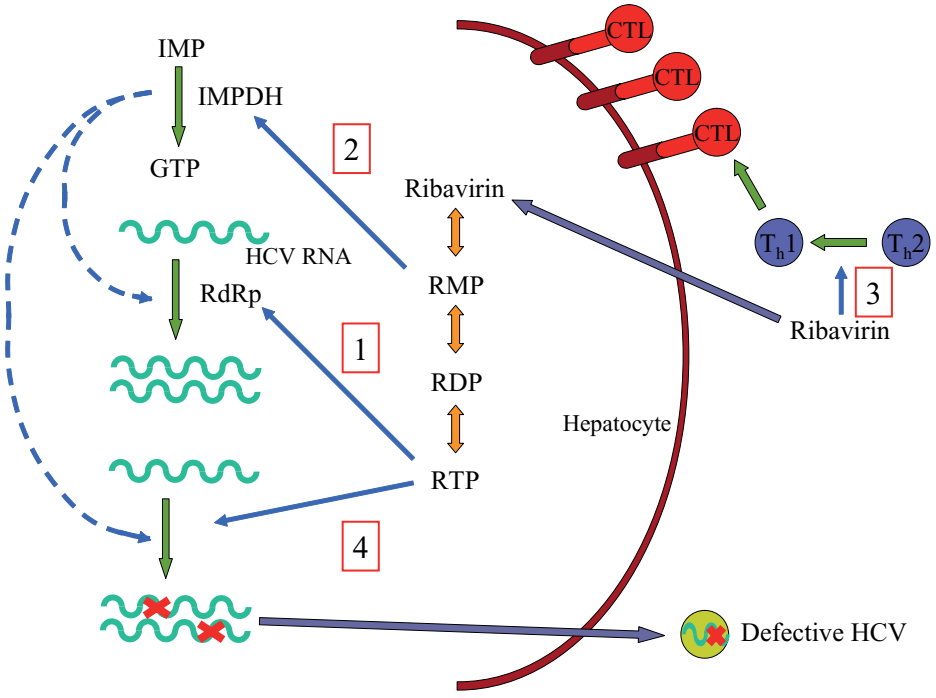

Figure 2. Proposed mechanisms for the antiviral activity of ribavirin against HCV: (1) direct inhibition of HCV replication, (2) inhibition of the enzyme IMPDH leading to GTP depletion, which in turn may lower viral production and/or facilitate greater incorporation of RTP in replicating RNA strands and hence enhance mutagenesis, (3) immunomodulation via a T_h2 to T_h1 shift in the immune response and (4) mutagenesis leading to defective progeny virions. T_h, T helper cells; CTL, cytotoxic T cell; RdRp, RNA-dependent RNA polymerase.

opposite ribavirin was found to be ~50000% faster than ribavirin incorporation opposite cytidine and uridine. RTP has also been shown to be incorporated into replicating HCV RNA strands, significantly inhibiting chain elongation [40]. Similarly, ribavirin has been found to weakly inhibit the activity of many viral polymerases [41–48]. With HCV, however, ribavirin concentrations in the range 40–150 µM, which are much higher than clinically achieved concentrations (~9 µM) [27], were required to observe the inhibition of polymerase activity [25, 40, 46–48]. Thus, the direct inhibition of viral polymerase activity by ribavirin is expected to have only a minor antiviral effect against HCV *in vivo*.

A second line of reasoning also suggests a limited anti-HCV role for the direct inhibition of viral replication by ribavirin. Following the onset of interferon therapy, a rapid first phase decline of plasma HCV RNA is observed for ~1–2 days [32] (Fig. 3). This decline is attributed to a reduction in production and/or release of new virions from infected cells due to interferon action [32]. Thus, if ribavirin were to inhibit viral replication, a similar decline in plasma HCV RNA would be expected following the onset of ribavirin therapy. Indeed, Pawlotsky et al. have recently observed that ribavirin does induce an initial viral load decline in a fraction of patients treated [49]. The magnitude of the decline, however, is significantly smaller than that induced by interferon. Interferon can induce a decline of several orders of magnitude (logs) [32, 49–51]. Ribavirin, in contrast, induced only ~0.5 log decline in HCV RNA, and this decline was transient; viral loads resurged to pretreatment levels in ~4 days [49]. The origins of this resurgence remain unclear. In addi-

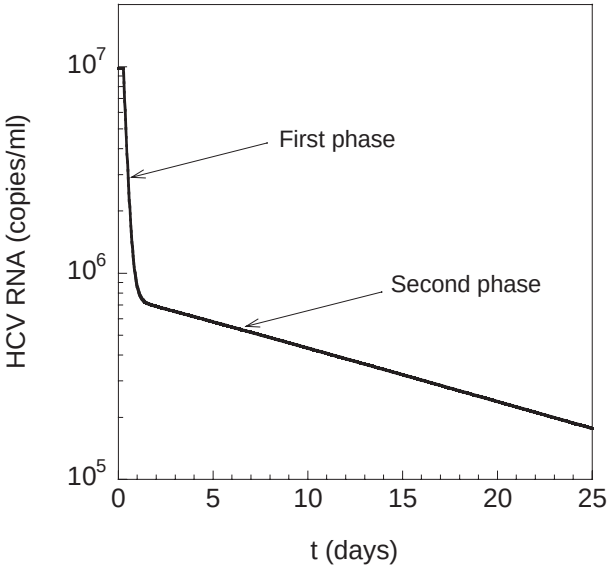

Figure 3. Typical two-phase profile of plasma HCV RNA decline in patients following the onset of interferon therapy.

tion, ribavirin is observed not to enhance the first phase decline in combination with interferon even when interferon effectiveness is sub-maximal [49–51]. Thus, only a minor role – if any at all – may be attributed to a direct antiviral action of ribavirin, such as inhibition of viral polymerase activity.

Inhibition of IMPDH

RMP can cause a depletion of intracellular GTP pools by competitively inhibiting the host enzyme IMPDH essential for the *de novo* synthesis of GTP [25, 52–57]. GTP depletion may influence viral replication in two ways. First, it may inhibit viral replication because of a lack of an adequate supply of GTP required for RNA synthesis. Second, it may promote the incorporation of RTP in place of GTP and thus increase the error rate during replication, contributing to the mutagenic activity of ribavirin (see below). Whether either of these pathways significantly contributes to the antiviral activity of ribavirin remains unclear. Ribavirin and other inhibitors of IMPDH, viz., mycophenolic acid and VX-497, inhibit HCV replication in the replicon system [17, 58, 59]. However, whereas addition of excess guanosine fully restores HCV replication rates with mycophenolic acid and VX-497, replication rates are only partially restored with ribavirin [48, 58, 59]. Thus, mechanisms other than the depletion of GTP via IMPDH inhibition appear to contribute to the antiviral activity of ribavirin. Perhaps, even with an adequate supply of GTP, incorporation of RTP in nascent RNA strands may occur and induce mutagenesis.

Immunomodulation

Several studies have suggested that ribavirin has immunomodulatory effects [17, 25, 51], such as the ability to induce a T_h2 to T_h1 shift in the immune response [60–62]. A T_h1 bias implies a stronger cellular rather than antibody response against infection. Indeed, stronger HCV-specific T cell responses have been observed in patients under combination therapy than in patients under interferon monotherapy [63], but this could be an effect of reducing viral load rather than an indication that ribavirin works via immunomodulation.

Ribavirin monotherapy does not lead to SVR [9–13]. In combination with interferon, ribavirin does improve SVR significantly over that with interferon monotherapy [14–16]. These observations suggest synergistic interactions between interferon and ribavirin. However, evidence of immunomodulation by ribavirin acting synergistically with interferon is lacking.

Loss of infected cells by immune-mediated killing is thought to underlie the second phase decline in HCV RNA (Fig. 3) in patients undergoing interferon-based therapy [32, 50, 51]. In some studies, ribavirin is observed to enhance this second phase decline [49, 51]. Hermann et al. have recently observed a third phase in some patients, starting somewhere between days 7 and 28 from the onset of therapy, in which HCV RNA decline is faster than in the second phase [51]. The third phase decline was faster in patients under combination therapy than under interferon monotherapy. Hermann et al. attribute this phase to a better restoration of the host immune response in the presence of ribavirin. Enhancement in T cell responses leading to a greater loss of infected cells is expected to increase HCV RNA decline in the second phase (and third phase if present) independent of interferon effectiveness. If ribavirin and interferon acted synergistically, as suggested above, then the enhancement in the second phase decline would increase with interferon effectiveness. In contrast, recent experimental observations suggest that the addition of ribavirin enhances the second phase slope but only when interferon effectiveness is low [28, 50, 51]. Thus, greater evidence is required to establish an antiviral role of ribavirin induced immunomodulation.

Mutagenesis

RNA viruses typically exist as a quasispecies – a collection of related but not identical genomes – due to their highly error prone replication mechanisms [64]. The existence of a quasispecies may provide the necessary diversity to the viruses and facilitate escape from specific host-immune responses and drug therapy. However, if the error rate during replication crosses a threshold, the quasispecies may be driven into an error catastrophe, resulting in a meltdown of genetic information [65–67]. Ribavirin is known to increase the error rate during viral replication by being incorporated in place of guanosine in nascent viral RNA strands [38, 39, 68]. Thus, ribavirin may potentially drive viruses to extinction by inducing an error catastrophe. Even if the increase in the mutation rate caused by ribavirin is inadequate to trigger a complete meltdown of genetic information, a higher mutation rate may render an increasing fraction of progeny virions defective by compromising their ability to infect new cells and/or replicate.

Several recent studies lend support to mutagenesis as the key mechanism of the antiviral activity of ribavirin [39, 68–74]. Crotty et al. have shown that mutagenesis can account for the entire antiviral activity of ribavirin against poliovirus [39]. Poliovirus lost more than 70% of its infectivity in one round of infection *in vitro* in the presence of 100 μM ribavirin, which increased the mutation rate from ~1.5 mutations/genome (wild type) to ~1.9 mutations/genome [39]. Higher doses caused greater mutation; at 400 μM ribavirin the mutation frequency increased to ~6.9 mutations/genome and at 1000 μM to ~15.5 mutations/genome [39]. A 9.7-fold in-

crease in the mutation rate caused a 99.3% loss in infectivity. With the HCV replicon system, ribavirin was shown to inhibit the ability of progeny subgenomic replicons to transfect new cells, although the replication rate remained unaffected [70, 72]. Ribavirin thus caused the production of defective progeny genomes. The specific infectivity of GB virus B, a close relative of HCV, was also reduced in the presence of ribavirin [72]. Indeed, by assuming that ribavirin renders a fraction of replicating HCV virions noninfectious, a recent model of HCV dynamics is able to explain quantitatively a number of observations of viral dynamics and long-term response rates following combination therapy with interferon and ribavirin [28].

Direct observations of an increase in HCV mutation rates in infected humans are still inconclusive [75–79]. Young et al. found that ribavirin modestly increased mutations in HCV in individuals under prolonged ribavirin therapy [76]. More recently, Asahina et al. found that ribavirin significantly enhanced the mutation rate in the NS5A and NS5B regions of HCV and that the former also correlated strongly with increased SVR in patients [78]. Others, however, have failed to observe an enhancement in the mutation rate induced by ribavirin [49, 77].

In summary, growing evidence favors mutagenesis as the key mechanism of the antiviral activity of ribavirin, but conclusive evidence is still awaited.

Pharmacokinetics

The antiviral effect of ribavirin has been observed to depend significantly on plasma concentration and dosage [49, 80–82]. Thus, along with the underlying mechanism(s), the pharmacokinetic properties of ribavirin may hold key links to the identification of improved therapeutic protocols. The pharmacokinetic properties of ribavirin have been studied extensively [27, 49, 80–87]. Ribavirin is typically administered orally in doses of 400, 500 or 600 mg twice daily. Following a single oral dose, the plasma concentration of ribavirin exhibits a three-phase profile – a rapid absorption phase, followed by a rapid distribution phase, and a long terminal elimination phase [27] (Fig. 4). Ribavirin is rapidly absorbed into circulation, transported actively by gastrointestinal N1 sodium-dependent nucleoside transporters in the proximal small bowel [88]. Following a 600 mg dose, the mean maximum concentration in plasma, C_{max}, was observed to be 782 ng ml^{-1} [85]. The mean time to the maximum concentration, t_{max}, was ~1.5 h [83–85]. The half-life of the distribution phase was ~3.7 h [27]. The terminal elimination phase was long with a mean final concentration time point following single dosing at ~100 h [85]. The elimination half-life was 79 h [85]. The mean area under the curve, AUC, was 13394 ng ml^{-1} h [85]. The AUC, remarkably, obeys a linear relationship with dosage over the dose range of 200 to 1200 mg [27].

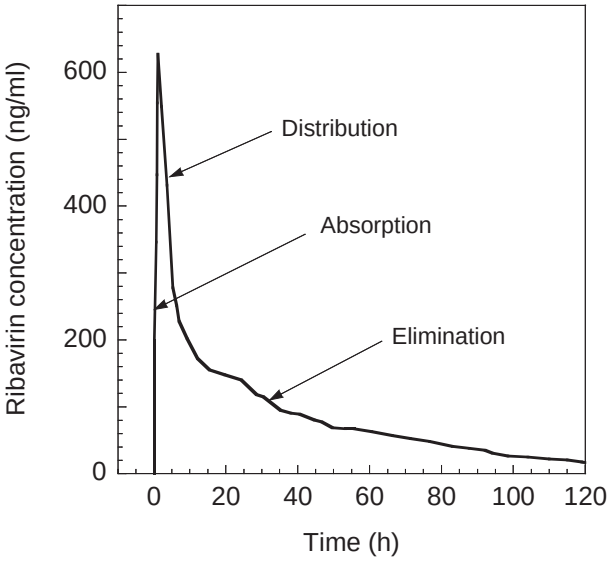

Figure 4. Three-phase pharmacokinetic profile of ribavirin concentration in plasma following a single oral dose (600 mg). The data is the average of 24 patients [27].

Following multiple dosing, ribavirin gradually accumulates in plasma, reaching a maximum asymptotic concentration in ~4 weeks [49, 85]. The mean asymptotic C_{max} following multiple doses of 600 mg twice daily was 3677 ng ml^{-1}, indicating a nearly 5-fold increase in C_{max} over that following a single dose [85]. The AUC following multiple dosing was 227867 ng ml^{-1} h [85]. Remarkably, the terminal elimination phase was elongated further following multiple dosing with a mean $t_{1/2}$ in the range 274–298 h, and ribavirin was detectable until 4 weeks following cessation of dose administration [27, 85].

Ribavirin is eliminated by metabolism as well as renal elimination, the latter accounting for about 5–15% of single dose elimination [83, 84]. Metabolism thus plays a key role in ribavirin elimination. The site(s) of ribavirin metabolism remain to be established. The pharmacokinetic profile of ribavirin remained unchanged between patients with healthy livers and those with hepatic dysfunction, suggesting that the liver is not a major site of ribavirin metabolism [84]. Further, about 85% of the orally administered drug was found to be absorbed, although the absolute bioavailability of ribavirin is only ~50% [86], which remains unchanged with hepatic dysfunction [84]. The gastrointestinal tract and not the liver thus appears to be the major site of first pass elimination [87].

Ribavirin does not bind to plasma proteins and is transported into cells by e_s nucleoside transporter molecules that are probably present on all cells in humans [89]. Ribavirin may thus be absorbed extensively into various cellular compartments. Khakoo et al. therefore suggest that

the long elimination phase of ribavirin might be due to the slow elimination from some 'deep compartment' [85]. Jarvis et al. have examined the transport of ribavirin into erythrocytes by e_s transporter molecules *in vitro* [89]. Ribavirin uptake was rapid; the intracellular ribavirin concentration rose linearly with time for short times (~30 s) and reached the extracellular concentration (~5 μM) in less than 1 min, at which value the intracellular concentration appeared to saturate. Ribavirin thus had a saturable influx mechanism with a mean Michealis-Menten like constant, K_m, of 440 μM at 22 °C.

Ribavirin is phosphorylated intracellularly by adenosine kinase into RMP but at a rate that is significantly lower than adenosine; the velocities of the phosphorylation reactions are similar for both adenosine and ribavirin, but K_m for ribavirin is 10000 times larger than for adenosine [26, 56]. RMP is further phosphorylated by nucleoside mono- and diphosphate kinases yielding RTP, the predominant intracellular metabolite of ribavirin [90]. RTP accumulates to very high concentrations in cells, reaching >100 μM in a few hours of incubation in a culture containing physiological concentrations of ribavirin [55]. Phosphorylation of ribavirin to RMP thus appears to be the rate-limiting step in the intracellular metabolism of ribavirin.

Depending on whether a cell contains a nucleus or is anucleate, ribavirin is reversibly or irreversibly phosphorylated inside the cell [27]. RTP appears to be rapidly dephosphorylated in nucleated cells when ribavirin is removed from cell culture. The dephosphorylation half-life is less than 2 h [91]. In erythrocytes, which are anucleate and lack dephosphorylation enzymes, RTP accumulates excessively. The e_s transporters cannot transport phosphorylated analogs of ribavirin [91]. Ribavirin is thus eliminated extremely slowly from erythrocytes and has a half-life of ~40 days, suggesting that removal is due to splenic hemolysis [83]. One consequence of this slow elimination might be the elongated terminal elimination phase of plasma ribavirin pharmacokinetics.

A second consequence of ribavirin accumulation in erythrocytes is the well-known side effect of ribavirin, hemolytic anemia [25, 83]. Due to the structural similarity between RTP and ATP, following sufficient accumulation, RTP may competitively inhibit ATP-dependent utilization in cells. The consequent effect on oxidative respiration is thought to decrease erythrocyte lifespan [92]. Thus, sustained ribavirin dosing can cause hemolytic anemia. The side effect is reversible, however, as erythrocyte counts recover following cessation of ribavirin administration [11, 12].

Standard mathematical models of drug pharmacokinetics, which typically include an absorption process and an elimination process of the drug in plasma, predict a two-phase evolution of plasma drug concentration following a single oral dose [93–95]. These simple models are thus unable to capture the three-phase pharmacokinetic profile of ribavirin [83], and more complex pharmacokinetic models need to be used. A further difficulty arises as the elimination half-life appears to change with multiple dosing [27]. Thus, parameters that describe single-dose pharmacokinetics may not be applicable to the multiple-dose case. Preston et al. [86] have employed two and three compartment models to fit experimental ribavirin plasma drug concentration profiles and find that the three compartment model provides a superior fit. However, what these compartments represent physically remains unclear. Currently, no mechanistic models of ribavirin pharmacokinetics exist that incorporate descriptions of drug absorption and elimination processes along with the known transport and metabolic processes specific to ribavirin.

Antiviral activity

Before HCV RNA testing was implemented in clinical practice, alanine aminotransferase (ALT) levels served as a surrogate marker of HCV infection and liver injury. This enzyme, which is present in the cytosol of hepatocytes, is generally present at low levels in the serum of people with normally functioning livers. With hepatic injury ALT leaks from the liver, causing an elevation of serum ALT. Interestingly, ribavirin has been observed to induce a significant decline in serum ALT levels [9–13]. With 24 weeks of ribavirin monotherapy, 29% of patients treated achieved normal ALT levels with an overall 55% of the patients experiencing some reduction in ALT [11]. The cause of this benefit of ribavirin remains unknown, although it would be consistent with ribavirin being anti-inflammatory and down-modulating cell-mediated immune responses. This type of effect of ribavirin is hard to reconcile with the notion that ribavirin induces a Th2 to Th1 shift, since such a shift would increase cell-mediated immune responses. Cessation of therapy raised ALT to abnormal levels in a majority of the patients [11, 12]. In combination with interferon, several patterns of ALT change have been observed that have been interpreted with viral kinetic models of interferon action with little regard to the presence of ribavirin [97]. The effects of ribavirin on ALT have not yet been the subject of modeling studies.

Ribavirin influences HCV RNA decline in subtle ways. Ribavirin monotherapy was recently found to induce a small, transient, early HCV RNA decline in a fraction of the patients treated [49]. Patients who exhibited this decline were observed to have higher plasma half-lives and concentrations of ribavirin than those who exhibited no decline. No patients exhibited a long-term response, however, in agreement with the observations of previous clinical trials of ribavirin monotherapy [9–13].

In combination with interferon, ribavirin does not influence the first phase decline of HCV RNA (Fig. 2). The

second phase decline, however, is enhanced by ribavirin in some cases but not in others [49–51]. Nonetheless, as mentioned above, a dramatic improvement in the long-term response rates over interferon monotherapy results from the addition of ribavirin [14–20].

Dixit et al. [28] have recently developed a mathematical model that quantitatively explains many of the effects of ribavirin on HCV RNA decline and long-term response rates in combination therapy with interferon. The model assumes that ribavirin renders a fraction of replicating virions non-infectious. Whether this is due to mutagenesis or otherwise does not alter model predictions. The following equations describe viral load evolution under combination therapy.

$$\frac{dI}{dt} = \beta T V_I - \delta I \tag{1}$$

$$\frac{dV_I}{dt} = (1-\rho)(1-\varepsilon) p I - c V_I \tag{2}$$

$$\frac{dV_{NI}}{dt} = \rho(1-\varepsilon) p I - c V_{NI} \tag{3}$$

Here, infectious HCV virions, V_I, infect target cells, T, at rate $\beta T V_I$ to form productively infected cells, I, which in the absence of therapy release new virions at rate p per cell. Interferon lowers p by a factor $(1 - \varepsilon)$, where ε is the effectiveness of interferon. Of the virions released, ribavirin renders a fraction ρ non-infectious, giving rise to the population V_{NI}. ρ is the effectiveness of ribavirin. Productively infected cells die at rate δ, and free virions are cleared from plasma at rate c. The total viral load is the sum $V = V_I + V_{NI}$.

The model predicts that ribavirin does not influence the first phase of viral load decline, but enhances the second phase decline in a dose-dependent manner provided interferon effectiveness, ε, is low (Fig. 5a). When ε is high, ribavirin does not influence the second phase decline either (Fig. 5b). These predictions are in agreement with experiments [49–51] and resolve the confounding observation that ribavirin influences the second phase decline in some cases but not in others. Model predictions quantitatively capture experimental HCV RNA changes in patients under interferon monotherapy and combination therapy [50]. Best-fit parameter estimates suggest that ribavirin enhances the second phase slope not by increasing the death rate, δ, of infected cells, but by diminishing their formation rate by lowering viral infectivity, which is again in contrast to the immunomodulatory role of ribavirin [28]. Using the distribution of δ across patients obtained by fitting patient data, the model allows calculation of the fraction of patients in whom plasma HCV RNA drops below detection (100 copies/ml) is fully eliminated (i.e., reaches less than one copy in the 15 l of fluid volume in the typical 70 kg patient) during the course of therapy and thus the fraction of patients that exhibit ETR or SVR, respectively. Model predictions quantitatively explain the observed ETR and SVR rates to interferon monotherapy and combination therapy (see, e.g., Fig. 6). With this predictive ability, the model may be applied to assess the outcomes of altered dosages, treatment patterns and durations, and thereby identify optimal treatment protocols. The model thus presents a framework for rational therapy optimization.

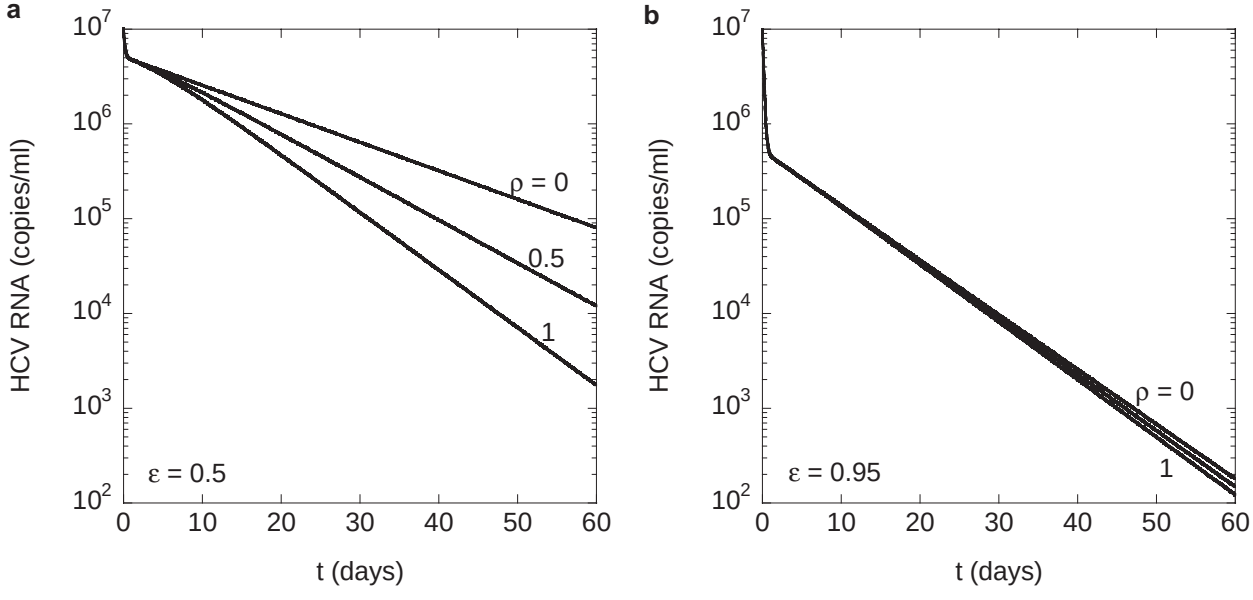

Figure 5. Calculations with the mathematical model of Dixit et al. [28] illustrating the effects of ribavirin on HCV RNA decline in combination therapy with interferon. (*a*) When interferon effectiveness is small, ribavirin enhances the second phase decline in a dose-dependent manner. (*b*) When interferon effectiveness is high, ribavirin has negligible influence on both the first and the second phases. Here, ε is the effectiveness of interferon, and ρ is the effectiveness of ribavirin.

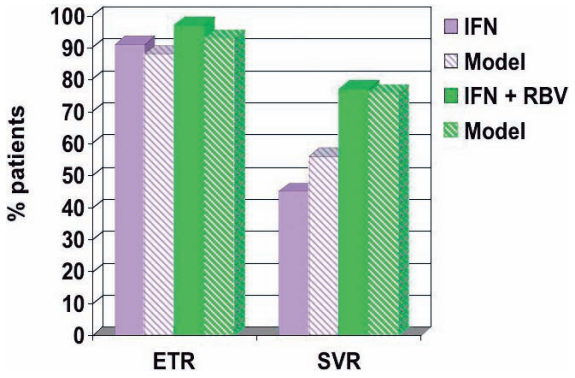

Figure 6. Comparisons of model predictions [28] and experimental observations [20] of long-term response rates (ETR and SVR) following 48 weeks of monotherapy with pegylated interferon and combination therapy with pegylated interferon and ribavirin. Note that the responses are compared in terms of the percentage of patients who completed therapy with the prescribed dosing regimen; patients who discontinued therapy or continued with reduced doses are ignored in the analysis (see [28] for details).

One limitation of the model is its inability to reconcile effects of ribavirin monotherapy with those of ribavirin under combination therapy. For instance, the poor long-term response rates under ribavirin monotherapy suggest that ribavirin effectiveness under monotherapy is small. The much higher ribavirin effectiveness required to explain enhancements in long-term response rates with combination therapy suggest synergy between ribavirin and interferon. The origins of this synergy remain to be established. A possible explanation may lie in the recently observed stimulation of interferon response elements by ribavirin, but this mechanism remains to be established for HCV [34]. Another possibility is that mutagenesis by reducing viral fitness may narrow the genetic diversity of HCV and hence reduce the ability of HCV to escape interferon-induced antiviral and immune pressures [17].

Summary

Recent studies have made rapid advances in elucidating the antiviral role of ribavirin. Growing evidence suggests that mutagenesis might be the underlying mechanism of action of ribavirin against many viruses. Concrete evidence establishing this mechanism, however, is still lacking. Ribavirin pharmacokinetics and the dose dependence of its antiviral activity have been characterized and present possibilities for improvement of treatment response. The peculiar three-phase pharmacokinetic profile of the plasma concentration of ribavirin, however, awaits mechanistic understanding. Against HCV, ribavirin monotherapy has little influence, but in combination with interferon response rates improve dramatically. Whereas viral dynamics models are able to explain the effect of ribavirin in combination therapy, the transient viral load decline under ribavirin monotherapy eludes our understanding. The origins of the synergy between ribavirin and interferon remain unknown. Therapy optimization, particularly for HCV, requires a thorough understanding of the antiviral activity of ribavirin, its mechanisms of action, pharmacokinetic properties, interactions with interferon, and toxicity and tolerance relationships. The future thus holds a lot in store for ribavirin research.

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