Ribavirin: pharmacology, multiple modes of action and possible future perspectives

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Ribavirin is a unique guanosine analog with broad-spectrum activity against many RNA and DNA viruses. In addition to its mutational properties, ribavirin exerts extensive perturbation of cellular and viral gene expression. Furthermore, recent advances indicate that the impact of ribavirin on divergent cellular and viral pathways may be concentration dependent. This review aims at providing an overview of the pharmacology and multiple modes of action of ribavirin as well as pointing to possible novel future uses.

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Ribavirin originally was synthesized as a guanosine analog [1] and was noted to exert unique broad-spectrum *in vitro* activity against both RNA and DNA viruses [2]. However, unlike many other antiviral nucleoside/nucleotide analogs, incorporation of ribavirin triphosphate does not appear to lead to chain termination [3]. Until recently, ribavirin was, together with IFN- α , a major therapeutic agent in HCV treatment [4–6], but ribavirin has also been indicated for respiratory syncytial virus infections [7], viral hemorrhagic fevers such as Lassa and Crimean–Congo [8,9], chronic hepatitis E virus infections [10], and many other severe, life-threatening viral infections [11]. Additionally, ribavirin has been tested against a wide range of infectious agents, including poliovirus [12] and influenza virus [11,13]. Despite ribavirin monotherapy only having modest effect on HCV RNA concentrations [14,15], the addition of ribavirin to IFN- α -based therapy markedly improved the likelihood of achieving a cure [4,5], also referred to as a sustained virologic response. Presently, the use of ribavirin in HCV therapy has diminished, but it continues to be recommended concomitantly with selected interferon-free, direct-acting antiviral treatments [16], especially for HCV genotype 3 infection. For example, among decompensated cirrhotic HCV patients enrolled in the ASTRAL-4 trial treated with sofosbuvir and velpatasvir, the highest sustained virologic response rate was observed among those also receiving ribavirin [17]. Thus, ribavirin likely will remain a part of the HCV therapeutic arsenal, especially in the setting of decompensated cirrhosis (Child–Pugh B or C), as protease inhibitors are contraindicated in this setting [16,18].

Pharmacology

Pharmacokinetic studies in healthy volunteers who received 150 mg of intravenous ¹³C₃-ribavirin followed after 1 h by a 400 mg oral dose showed that ribavirin had a mean bioavailability of 52% $\pm$ 22%, and a mean half-life of 37 $\pm$ 14 h [19]. Ribavirin has a large distribution volume, an elimination that is dependent on renal function, and thus requires more than 4 weeks to reach steady-state concentrations [20–22]. Upon cellular entry, ribavirin is activated by intracellular phosphorylation into mono-, di- and triphosphates. *In vitro* studies have shown that polyphosphorylation irreversibly entraps the ribavirin in the erythrocytes, resulting in intracellular levels in excess of 550 μ M, as compared with plasma concentrations of 15 μ M [23].

At steady state, which often occurs after more than 4 weeks of treatment in patients with normal renal function receiving weight-based ribavirin dosing for HCV infection, that is, 1 g (<75 kg bodyweight) or 1.2 g daily ($\geq$ 75 kg bodyweight) divided into two oral doses, plasma concentrations of ribavirin of approximately 8–12 μ mol/l (2–3 μ g/ml) are achieved [15]. However, it should be noted that although the optimal target trough concentration

of ribavirin remains unclear, toxicity increases dramatically at plasma concentrations exceeding 15 $\mu\text{mol/l}$ [24,25]. Despite this, considerably higher ribavirin dosing is recommended for many life-threatening viral infections, for example, for the treatment of Lassa hemorrhagic fever [26].

Shortening time to steady state

In a pilot study (RibaC), 2 weeks of ribavirin double dosing (i.e., 26 mg/kg/day orally instead of standard 13 mg/kg/day for HCV genotype 1 infection), resulted in high ribavirin plasma concentrations, but regrettably also in greater hemoglobin decline [15]. The results of this study suggest that 1 week of double dosing (followed by standard dosing) may suffice to achieve steady state as well as decrease the risk of anemia.

Ribavirin in renal impairment

Ribavirin is mainly eliminated by the kidneys, and the pharmacokinetics of ribavirin are substantially altered in patients with renal impairment [27]. Despite this, many patients with renal failure and/or ongoing hemodialysis have been successfully treated with ribavirin [21]. A prerequisite, however, for the use of ribavirin in this setting is the initiation of therapy with a reduced dose, dependent upon the degree of kidney impairment [24,28], in addition to continuous monitoring of plasma ribavirin concentrations and hemoglobin levels.

Adverse effects

The major side effect of ribavirin is hemolytic anemia, with a mean decrease in hemoglobin of approximately 20 g/l during treatment [24]. This hemolysis is hypothesized to be secondary to oxidative stress induced by depletion of adenosine triphosphate (ATP) in erythrocytes [29,30]. Because of the risks associated with anemia in patients with a history of or ongoing heart disease, ribavirin is contraindicated in these patients [24]. Aside from anemia, ribavirin is also associated with increased risk of itching, rash, cough as well as neuropsychiatric side effects, for example, insomnia [28].

Teratogenicity

In light of the potential teratogenicity demonstrated in all animal models studied, ribavirin is contraindicated during pregnancy and breastfeeding. Avoidance of pregnancy for 4 months after ribavirin exposure for treated women and for 7 months for female partners of treated men is therefore recommended, although preliminary analysis of the 'Ribavirin Pregnancy Registry' established in 2003 has not been able to demonstrate any clear evidence for human teratogenicity for ribavirin [31].

Impact of inosine triphosphate pyrophosphatase on ribavirin

Naturally occurring variants of the ITPase gene (*ITPA*) associated with reduced enzymatic activity, found in approximately a third of humans, have been demonstrated to protect against ribavirin-induced hemolytic anemia during ribavirin therapy in combination with peg-IFN- α [32,33] as well as with direct-acting antiviral therapy [34] for chronic HCV infection. Additionally, reduced ITPase activity has also been associated with improved efficacy of interferon- α and ribavirin therapy for HCV genotype 2 or 3 infection, mediated by a reduced relapse risk, in spite of lower ribavirin plasma concentrations [32]. Recently ribavirin triphosphate (RTP) has been reported to be dephosphorylated intracellularly to ribavirin monophosphate (RMP) by ITPase [35]. As ribavirin and RMP, but not RTP, can be transported across the plasma membrane through specific transporter proteins (ENT1, ENT2, CNT2, MRP4 and MRP5) [23,36–39], it is not surprising that reduced ITPase activity is associated both with higher intracellular RTP levels [35] and lower plasma ribavirin concentrations (Figure 1) [32].

Modes of action

The precise molecular mechanism underlying the action of ribavirin on HCV infection remains unclear, but several mechanisms of action have been proposed and their impact may be ribavirin concentration dependent:

Inhibition of inosine monophosphate dehydrogenase & effects on intracellular nucleotide concentrations

One of the first suggested mechanisms of action of ribavirin was the potent competitive inhibition of inosine monophosphate dehydrogenase (IMPDH) by RMP leading to reduced guanosine monophosphate biosynthesis by the diminution of the conversion of inosine monophosphate to xanthosine monophosphate, with subsequent

Figure 1. Modes of action of ribavirin, ribavirin monophosphate and ribavirin triphosphate on cellular functions (red) and viral functions (blue). At lower concentrations (10 μM), RMP inhibits inosine monophosphate dehydrogenase causing GTP depletion with secondary effects on mRNA-capping, G-protein signaling, ribosomal functioning, immune modulation (e.g., favoring Th1 over Th2 responses), etc. RTP may directly inhibit viral RdRp. At higher concentrations ($\geq 100 \mu\text{M}$), RTP causes mutations in the viral genome. RTP can be dephosphorylated to RMP by ITPase. Ribavirin can enter erythrocytes, which may lead to hemolytic anemia. RMP: Ribavirin monophosphate; RTP: Ribavirin triphosphate.

intracellular depletion of GTP [40]. This inhibition of IMPDH has been observed to occur even at relatively low ribavirin concentrations (10 μM ribavirin), and leads to marked changes in the balance of intracellular nucleotide concentrations [35]. These alterations in the nucleotide pool in cells may secondarily have major impact on host cell and viral gene expression as well as on viral replication [41–45].

Inhibition of mRNA capping

Eukaryotic mRNAs and many viral mRNAs contain extensive modification on the 5' end (known as the 'five-prime cap'), often utilizing guanine which is methylated in the 7-position, as this is essential for the stability and efficient translation of mRNA. Thus, RNA capping has major secondary impact on the translation of both viral and host cell mRNAs. Interestingly, RTP reportedly acts as a competitive inhibitor for the capping of mRNAs subsequently leading to impaired translation [46] by forming a covalent RMP-capping enzyme intermediate in place of the normally observed guanosine monophosphate-enzyme intermediate [47]. This observation, however, has been disputed [48].

Impact on host cell gene expression, inflammation & immunomodulation

Aside from being an essential purine needed for RNA synthesis during transcription, GTP also acts as a source of energy used in protein synthesis as well as gluconeogenesis [49]. For example, during the elongation stage of translation, GTP is essential for the binding of a new amino-bound tRNA to the A-site of the ribosome as well as

for the translocation of the ribosome toward the 3' end of the mRNA [49]. Additionally, GTP is necessary for signal transduction in particular with G-proteins, in second-messenger mechanisms [49].

Thus, in light of the above-mentioned importance of maintaining adequate intracellular levels of GTP, it is understandable that ribavirin impacts on host cell and viral gene expression both *in vivo* [42,43] and *in vitro* [44,45], as well as on HCV-specific immune responses by modulating the TH1/2 subset balance [50], and enhancing TH1 immune response and IL-12 levels [41]. Additionally, clinical HCV studies demonstrate that ribavirin monotherapy downregulates the expression of interferon-stimulated genes in addition to reducing systemic concentrations of liver enzymes [14] and IP-10 (also known as CXCL10) [15] in spite of only modest impact on viral levels. Similarly, ribavirin administration in mice infected with influenza virus significantly attenuated respiratory immune responses, as well as secretory and total IgA mucosal responses [51].

Inhibition of viral RdRPs

RTP has been reported to competitively inhibit the influenza virus RNA polymerase, with respect to ATP and GTP, whereas RMP has no such observable effect [52]. However, for HCV there is conflicting data regarding the impact of ribavirin on the RdRP with reports of both no direct inhibition [53], as well as observations that ribavirin containing RNA templates can cause a significant blockage of RNA elongation [54].

Enhancement of viral mutagenesis

It has been suggested that ribavirin enhances viral mutagenesis leading to error catastrophe by incorrect substitution of RTP for GTP [12,54,55] into viral RNA as most viral RdRPs lack proofreading, although this mechanism has been disputed for some viruses [56]. For example, for poliovirus, a 9.7-fold increase in mutagenesis following ribavirin treatment resulted in 99.3% loss in infectivity [12]. Unlike GTP, RTP has ambiguous base-pairing capacity and can form two hydrogen bonds with uridine triphosphate (UTP) or cytidine triphosphate (CTP) with equal efficiency [57], leading to a subsequent increase in G-to-A and C-to-U single nucleotide variations throughout the entire HCV open reading frame [58]. Recent *in vivo* studies indicate that similar ribavirin-induced mutagenesis occurs in hepatitis E virus [59] and the tamarin GB virus B [60]. Interestingly, the effects of ribavirin on *in vitro* HCV mutagenesis are observed at relatively high concentrations of ribavirin ($\geq 100 \mu\text{M}$) [35].

Conclusion

Ribavirin is a unique guanosine analog with effect against a broad range of viruses, and there is now increasing support for the notion of multiple mechanisms of action for its antiviral effect [61]. These differing modes, however, may vary across the ribavirin concentrations achieved as well as the virus studied. In the future ribavirin may have novel indications, especially when targeting both viral and host cell factors is desired. In light of the relatively long half-life of ribavirin, resulting in a considerable time to steady state when administered orally, this may be most appropriate in the setting of chronic rather than acute viral infections.

Future perspective

As ribavirin is unique in its broad antiviral effect, it likely will remain a potential drug for emerging viral diseases. It is also very possible that novel indications will materialize in the coming 5–10 years, especially for viral infections where targeting both viral and host responses is appropriate. In light of the extended time until achievement of steady state ribavirin concentrations when administered orally, this may be most appropriate in the setting of chronic viral infections where other therapeutic options are limited. Moreover, ribavirin is increasingly being studied as a therapeutic option in cancer. Currently, there are ongoing studies registered in ClinicalTrials.gov evaluating ribavirin in conjunction with follicular and mantle cell lymphoma, tonsil and/or tongue squamous cell carcinoma, and human papillomavirus-related malignancies.

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Executive summary

Ribavirin is a unique guanosine analog with broad-spectrum activity against many viruses including:

- Hepatitis C virus.
- Hepatitis E virus.
- Respiratory syncytial virus.
- Lassa virus.
- Crimean–Congo hemorrhagic fever virus.
- Poliovirus.
- Influenza virus.

Pharmacology

Ribavirin:

- Has a long half-life resulting in more than 4 weeks to reach steady state ribavirin concentrations when administered orally in healthy individuals. Double dosing for 1 initial week may result in faster achievement of steady state.
- Has a large distribution volume.
- Has an elimination that is dependent on renal function requiring dose reduction in patients with renal impairment.
- Has adverse effects including hemolytic anemia, itching, rash, cough and insomnia.
- Is teratogenic.
- Is contraindicated in heart disease, pregnancy and breastfeeding.
- Ribavirin triphosphate is dephosphorylated to ribavirin monophosphate by inosine triphosphate pyrophosphatase. Reduced inosine triphosphate pyrophosphatase activity results in decreased anemia, lower plasma but higher intracellular ribavirin concentrations, and improved HCV therapeutic outcome.

Modes of action

- Inhibition of inosine monophosphate dehydrogenase (IMPDH) resulting in intracellular GTP depletion occurs at lower ribavirin concentrations (10 μ M ribavirin). Reduced intracellular GTP secondarily may result in:
 - Inhibition of mRNA capping.
 - Impact on host cell gene expression, inflammation and immunomodulation.
- Inhibition of viral RdRPs.
- Enhancement of viral mutagenesis, which occurs at higher ribavirin concentrations *in vitro* (≥ 100 μ M), by means of the incorrect substitution of ribavirin triphosphate for GTP. Ribavirin triphosphate can form two hydrogen bonds with UTP or CTP with equal efficiency, resulting in an increase in G-to-A and C-to-U single nucleotide variations.

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