

Review Article

Ribavirin at the Era of Novel Direct Antiviral Agents for the Treatment of Hepatitis C Virus Infection: Relevance of Pharmacological Monitoring

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Ribavirin is often used for the treatment of hepatitis C virus (HCV) infection. Although its mechanisms of action remain to be clearly elucidated, ribavirin plays a beneficial role for achieving virological response and decreasing the rate of virological relapse after treatment cessation. However, ribavirin may induce side effects leading to early treatment discontinuation. Among them, hemolytic anemia is the most frequent and results from intraerythrocyte accumulation. Pharmacological studies have shown that early ribavirin exposure assessed by the area under the curve (AUC) at day 0 and ribavirin trough concentration during the first three months of therapy were correlated with sustained virological response (SVR). These studies highlighted the relevance of ribavirin pharmacologic monitoring and early dose adaptation during therapy. Although the role of ribavirin within new direct acting antiviral (DAA) combinations will probably decrease in the future, its potential benefit in difficult-to-treat patients such as patients with severe hepatopathy or patients who failed triple therapy including patients with multiresistance will need to be further investigated.

1. Introduction

Ribavirin (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a nucleotide analogue of guanosine and a broad-spectrum direct antiviral agent (DAA). Ribavirin was discovered 30 years ago and is efficient *in vitro* and/or *in vivo* against several RNA or DNA viruses [1–3]. This DAA is mostly used for hepatitis C virus (HCV) treatment, severe human respiratory syncytial-virus (RSV) infection, some hemorrhagic fevers, and more recently for immunosuppressed patients infected by hepatitis E virus (HEV) [4]. Partly based on the authors' own work [5–13], this paper focuses, in the context of HCV infection, on the ribavirin

mechanism of action, its efficacy according to different therapeutic schedules, and its side effects and toxicity. Since clinical and pharmacological data suggest that an adequate and early exposure to ribavirin improves virological response, the relevance of ribavirin pharmacological monitoring in different patient populations and in different therapeutic situations including new promising treatment strategies is also discussed.

2. Pharmacological Data

After a single oral dose, three different phases may be distinguished in ribavirin plasma concentration: a quick absorption

phase with a mean time of 1.5 hours to reach the maximum concentration (C_{max}), a quick distribution phase (half-life around 3.7 hours), and a long elimination phase with a last measurable concentration around 100 hours after intake [14, 15]. Ribavirin is assimilated at 90% with a N1 nucleoside-transporter active mechanism in the proximal small intestine. Its bioavailability is between 45% and 65% with an important variability within and between individuals that can reach 30% after an oral single-dose [16]. Equilibrium state is reached after 4 weeks of multiple dosing and the half-life is 300 hours.

Ribavirin is then carried to every part of the organism and is phosphorylated in the intracellular medium [13]. Ribavirin triphosphate is the major intracellular metabolite. Extracellular transport of ribavirin needs a dephosphorylation process. In erythrocytes, extracellular transport of ribavirin is not possible due to the absence of this dephosphorylation process which explains its intracellular accumulation. Half-life of ribavirin in erythrocyte is 40 days. Its elimination takes place with splenic hemolysis. Renal excretion of ribavirin and its associated metabolites represent only 40% of its total clearance [17].

Bruchfeld et al. [18] studied ribavirin pharmacokinetics in HCV patients with renal insufficiency. In a study on 63 patients, the authors showed that ribavirin clearance was linearly dependent on renal function with a small nonrenal clearance dependent on body weight and age. Estimated glomerular filtration rate (eGFR) was a better predictor of ribavirin clearance than body weight alone. However, the effect of renal function on ribavirin clearance was only observed for patients with a creatinine clearance under 34 mL/min. Therefore, prescription of ribavirin with or without interferon (IFN) should be adapted to weight but also to patient's renal function [19, 20]. In this Swedish work, the authors proposed a ribavirin-dosing schedule based on eGFR and body weight to reach an intended target concentration and concluded that follow-up of concentrations was crucial in patients with renal insufficiency. Other studies have demonstrated that ribavirin clearance was influenced by weight, age, gender, and creatinine but these cofactors could only explain 27 to 40% of interindividual variability [18, 21]. Metabolism mostly explains mechanisms of ribavirin clearance but sites of clearance remain unknown. Liver could probably play a role although Glue et al. showed that liver failure had no impact on ribavirin clearance. Only mean C_{max} and ribavirin concentration profiles between 0 and 6 hours were different between the three groups of patients (normal liver function, moderate liver failure, and severe liver failure) [22]. The gastrointestinal tract is the potential candidate for the major site of clearance [14]. Finally high fat meals can increase ribavirin bioavailability by 46% compared to fasted state [23].

3. Mechanism of Action

Ribavirin mechanism of action is still not perfectly elucidated (Figure 1). Ribavirin has an antiviral and immunomodulatory effect and several hypotheses for its antiviral effects have been proposed.

Feld et al. [24] suggested that ribavirin could increase IFN-sensitive target genes activity. A lead-in phase of ribavirin could also promote the expression of these genes that transform cells in more IFN-sensitive cells by increasing the production of endogenous IFN. Similar results were observed *in vitro* with the respiratory syncytial virus (RSV) [25].

We recently showed that ribavirin could interact *in vivo* and *in vitro* with IFN genes expression [10]. We know that activation of these genes before treatment is associated with a poor-probability of response to IFN [26–28]. *In vivo*, ribavirin treatment reduces the mRNA levels of a large number of interferon stimulated genes (ISGs), particularly those found upregulated in nonresponders to PEG-IFN/ribavirin [10]. This effect was confirmed *in vitro* in primary human hepatocytes and differentiated HepaRG cells, in which exposure to ribavirin alone resulted in downregulation of a large set of ISGs. Ribavirin, by reducing this activation, could restore the possibility of an endogenous or therapeutic IFN-response in HCV patients. This ISGs downregulation correlated with liver biochemical response, with a sharper inhibition observed in patients showing ALT normalization following RIBAVIRIN treatment. This mechanism explains the relevance of ribavirin in therapeutic combinations with or without IFN and could explain the biological and histological effects observed in ribavirin monotherapy [12].

These results suggest that a sufficient and early exposition to ribavirin (absorption and distribution) could stimulate IFN mechanisms of action and induce a very early viral load decrease.

Another possible mechanism of action has been proposed. Some studies [29, 30] suggested a dose-dependent mechanism of action on the second slope of viral load decrease among patients with a low IFN efficacy. The authors suggested that ribavirin does not increase mortality rate of infected cells but rather decreases viral infectivity through viral mutagenesis. Ribavirin triphosphate could incorporate in DNA instead of guanosine triphosphate and could in this way increase the replication error rate including lethal mutations of the virus [31]. However, these so-called “error catastrophes” were not confirmed by other studies [9, 11].

This mutagenesis process could be weak during monotherapy. However, in presence of IFN, viral production could decrease and relative ribavirin concentration in each viral genome being replicated in infected cells could increase, hereby increasing the mutation rate. In HCV-replicon, ribavirin can inhibit infection of new cells by new produced genomes although replication rate is still the same [29, 30]. However, another study seems to contradict this hypothesis [32].

Two other hypotheses are today less and less considered. Ribavirin could inhibit HCV RNA polymerase by a direct antiviral action as demonstrated in the replicon model. A study by Pawlotsky et al. [33] showed a decrease of the initial viral load of about 0.5 log in some patients treated by ribavirin monotherapy. This decrease was shown to correlate with ribavirin plasma concentration [34]. The other hypothesis is that ribavirin could promote a decrease of

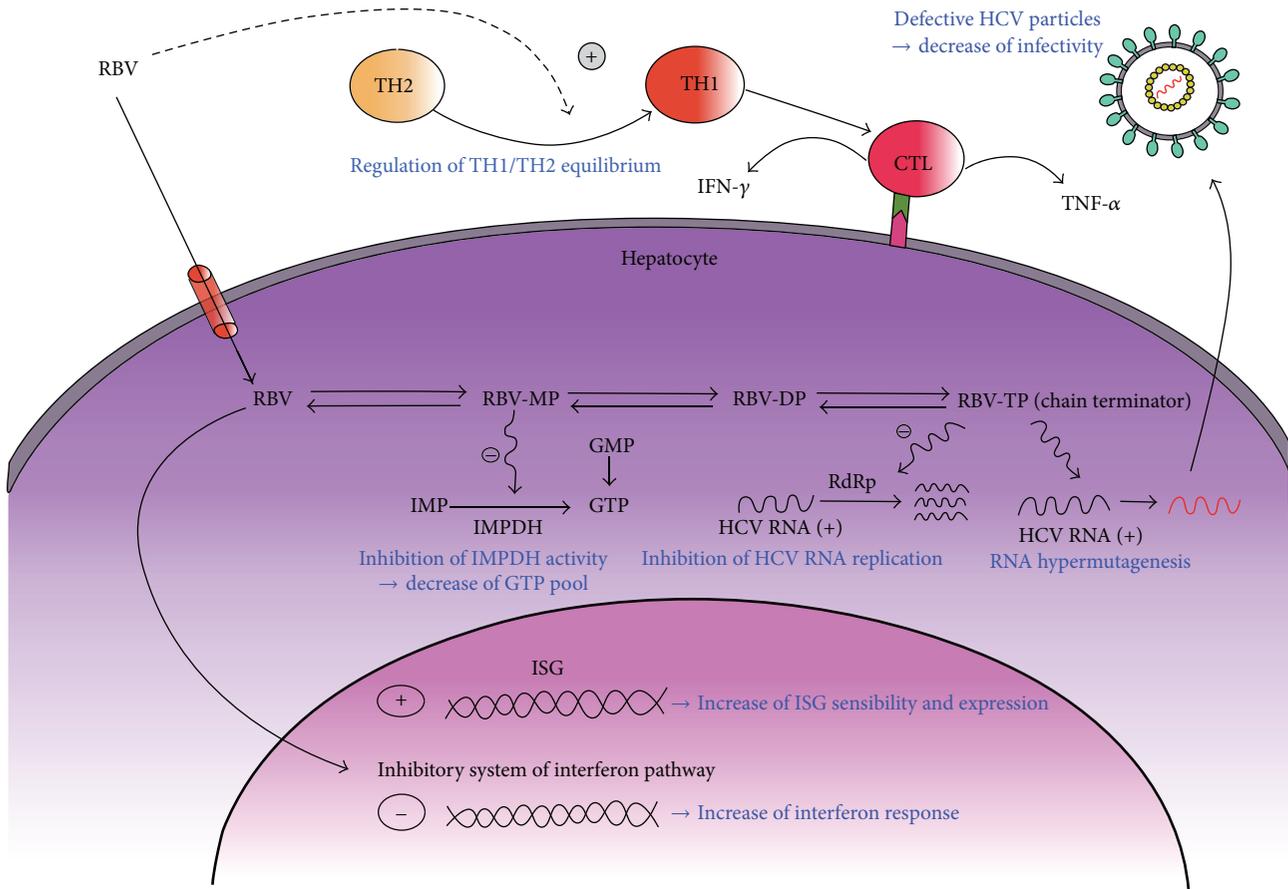


FIGURE 1: Ribavirin mechanism of action.

guanosine triphosphate (GTP) pool. Moreover ribavirin 5'-monophosphate is a strong inhibitor of IMP-dehydrogenase [35], hereby impairing de novo-GTP synthesis necessary for viral replication [36, 37].

Finally, ribavirin also has an immunomodulatory role in the positive regulation of auxiliary TH1/TH2 T-lymphocytes. This property could explain the decrease of liver transaminase level during treatment.

4. Side Effects and Haematological Toxicity

Ribavirin induces side effects responsible for 3 to 11% of early treatment cessation that may reach 35% among cirrhotic patients [12]. These side effects are mostly reversible at the end of treatment. Ribavirin induces in particular allergic reactions such as chronic dry cough or pruritus but the most frequent side effect is haemolytic anaemia due to intraerythrocyte accumulation. During pegylated (PEG)-IFN/ribavirin combination therapy, 25% of patients develop anaemia with haemoglobin level <10 g/dL and 13% of patients need ribavirin dose modification [38, 39].

This ribavirin-induced anaemia seems to be correlated with ribavirin plasma concentration rather than with the ratio dosage/weight [40]. A Japanese study [41] reported a

correlation between the decrease of haemoglobin level and ribavirin plasma concentration but no association between severity of anaemia and initial haemoglobin level was reported.

Several independent studies reported an association between inosine-triphosphate-pyrophosphate (ITPA) genetic polymorphism and a protective effect against ribavirin-induced anaemia. ITPA promotes inosine-triphosphate (ITP) hydrolysis in inosine-monophosphate (IMP). Several ITPA gene polymorphisms associated with a defect in ITPA-activity were identified. The induced ITP accumulation could substitute guanosine triphosphate depleted by ribavirin during ATP-synthesis process. In erythrocytes, the depletion of ATP induced by ribavirin gives rise to oxidative stress and erythrocyte lysis. A reduced activity of ITPA could thus have a protective effect against ribavirin-induced anaemia. During combination therapy, lower grades of anaemia were observed in patients with minor variants of ITPA and also in patients under triple therapy with boceprevir or telaprevir [42–44].

Moreover, haptoglobin phenotype was shown to be a determinant factor in ribavirin-induced haemolytic risk [45]. Although the exact mechanism remains speculative, the authors showed that the Hp 1-1 phenotype was associated with a higher hemoglobin drop between week 0 and week 8 of ribavirin exposure.

5. Efficacy

5.1. Efficacy during Monotherapy. After several months of monotherapy, ribavirin induces a progressive normalization of transaminases in 30 to 50% of cases with a constant relapse at the end of treatment [46]. Histological benefit of long-term regimens has been suggested but has not been confirmed in large-scale studies [12].

In a study by Bonaventure et al. [5], effects of ribavirin monotherapy were evaluated among 83 patients treated for a mean duration of 60 months. Results showed a low antiviral efficacy (median decrease of HCV RNA of 0.2 log), a biochemical response in 60% of cases, an histological improvement with 44% of activity score improvement whatever the treatment response, and a 24% fibrosis score improvement among patients with normalized transaminases.

5.2. Efficacy during PEG-IFN/Ribavirin Combination Therapy. In 1994, Brillanti et al. showed promising results of PEG-IFN/ribavirin combination therapy [47]. Ribavirin became afterwards the major drug of anti-HCV IFN-based treatments [48, 49] and later on of PEG-IFN based treatments [38, 39, 50]. Combination therapy increased sustained virological response (SVR) rates by 25–30%. This positive effect of ribavirin was associated with a reduction of relapse-rate after the end of treatment. It was suggested that ribavirin prevented relapse by significantly increasing the second slope of HCV-RNA decrease [51].

5.3. Efficacy during Triple Therapy. Recent studies combining DAA with ribavirin also suggested a positive effect of ribavirin in such treatment strategies. The first study reporting a potential effect of ribavirin involved the RG1626 nucleoside inhibitor and showed a synergic effect of ribavirin [52].

The benefit of ribavirin in protease inhibitor (PI)-based treatments was reported in an *in vitro* study [53] and in three large phase-II studies: PROVE II [54] PROVE III [55] and SPRINT-1 [56]. Patients that did not receive ribavirin in PROVE-studies and those with a low ribavirin dosage in SPRINT-1 study had a higher probability of viral breakthrough or relapse and a lower SVR rate. These studies show that standard ribavirin dosage is necessary to get the best response to a first generation PI-based treatment.

5.4. Benefit in IFN-Free Regimens. The usefulness of ribavirin when associated with DAA in IFN-free regimens is still unclear. Some studies showed that IFN-free treatments based on protease or polymerase inhibitors were more efficient when combined with ribavirin [57, 58]. These studies suggested a benefit of ribavirin when combined with DAA, particularly when associated drugs have a low genetic barrier [59].

Three new DAA (sofosbuvir, daclatasvir, and simeprevir) are now available in western countries and are the basis of new IFN-free combinations. The sofosbuvir/ribavirin combination during 12 weeks is today the standard treatment for genotype 2 infections, with or without cirrhosis, with SVR rates above 90% [60, 61]. This combination may also

be proposed during 24 weeks in genotype 3 patients or in genotypes 1 or 4 patients in whom PEG-IFN cannot be used [62]. The ribavirin dose seems to impact virological response and it was shown that low doses (600 mg/day) were associated with lower virological response than weight-based doses (48% versus 68%) [63].

Two recent studies assessed the efficacy of a sofosbuvir/simeprevir or sofosbuvir/daclatasvir combination with or without ribavirin in genotype 1 patients with or without cirrhosis. The addition of ribavirin to the sofosbuvir/simeprevir or sofosbuvir/daclatasvir 12–24 week combinations did not improve SVR and was more frequently associated with side effects [64–66]. These results seem to indicate no clear-cut benefit of ribavirin when added to DAA combinations containing a nucleoside polymerase inhibitor with high genetic barrier and another antiviral. However, some preliminary results indicate that such combinations may not be sufficient in difficult-to-treat patients such as genotypes 1 or 3 decompensated cirrhotics and suggest that the addition of ribavirin could improve virological response [67]. The role of ribavirin within new DAA combinations will probably decrease in the future. However, its potential benefit in difficult-to-treat patients (patients with severe hepatopathy or multiresistance) will need to be further investigated.

6. Ribavirin Monitoring

Current clinical and pharmacodynamic data suggest that an adequate and early exposure to ribavirin increases SVR and decreases relapse rates.

The main purpose of ribavirin pharmacologic monitoring is to potentially adapt ribavirin dosage. Variability of ribavirin plasma concentrations in relation with the interindividual variability of pharmacokinetic parameters has to be controlled and reduced with ribavirin dosage. Moreover a double relation exists between first ribavirin plasma concentration and antiviral efficacy and secondly between ribavirin plasma concentration and toxicity. Nevertheless, direct correlation between ribavirin dosage and ribavirin plasma concentration does not exist [15]. Weight, gender, age, diet, creatinine level, and other unknown pharmacogenetic factors are probably involved in ribavirin pharmacokinetics [21].

6.1. Plasma or Serum. Most studies about ribavirin focus on ribavirin plasma concentration. However, *in vitro* ribavirin plasma concentration decreases over time if blood sample is not analysed immediately after collection. This decrease is due to an intraerythrocyte transport of ribavirin with the help of specific equilibrative transporters. This plasma decrease is around 30% after one hour of storage at room temperature and around 60% after three hours. The most plausible hypothesis is that ribavirin is still phosphorylated in erythrocytes *in vitro* inducing this decrease of plasma ribavirin.

Preanalytical phase of ribavirin plasma concentration measures was thus standardised and ribavirin trough plasma concentration is measured from a blood sample stored in ice and centrifuged within 15 minutes after collection.

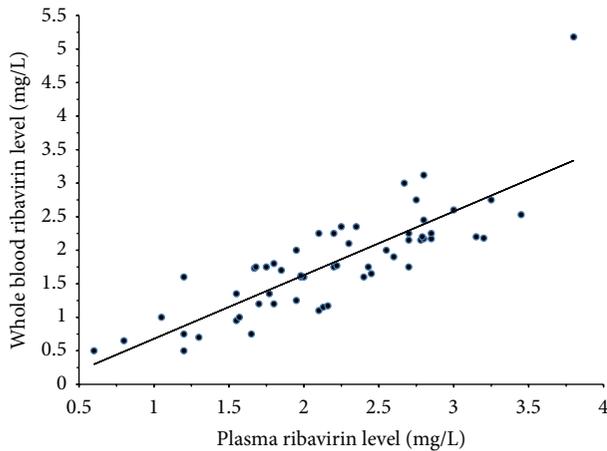


FIGURE 2: Ribavirin concentration in plasma and in whole blood of 57 patients treated by pegylated interferon/ribavirin (correlation coefficient: 0.841, $r^2 = 0.71$, 95% confidence interval 0.743–0.904).

6.2. *Erythrocyte*. Measure of intraerythrocyte ribavirin concentration was suggested to be more relevant [22]. The authors showed that early intraerythrocyte accumulation of ribavirin was higher among patients who achieved SVR. However, irreversible accumulation of ribavirin triphosphate is probably not a good turn-over marker of the drug in the other target cells such as hepatocytes, where the activation of ribavirin in triphosphate metabolite is reversible. Currently, no scientific proof shows that ribavirin intraerythrocyte concentration is better than plasma concentration.

6.3. *Whole Blood*. We measured ribavirin concentration in frozen whole blood samples immediately after blood sample collection in order to reduce the variability of concentrations during the preanalytical phase. Ribavirin concentrations in frozen whole blood samples were compared with ribavirin plasma concentrations. This comparison was first performed in a pilot study of 57 HCV patients receiving PEG-IFN/ribavirin combination therapy. Mean ribavirin concentration in whole blood (1.89 ± 0.75 mg/L) was equivalent to mean ribavirin plasma concentration (2.17 ± 0.67 mg/L) (Figure 2).

7. Correlation AUC/Trough Concentration/SVR in Combination Therapy

First attempts of pharmacokinetics/pharmacodynamic study in the literature are based on single-dosage strategy. Based on a study of 1367 patients, Jen et al. concluded in their response model that ribavirin serum concentration at week 4 (W4) was a predictive factor of SVR. However, this factor was less predictive of SVR than genotype and viral load [68]. Nevertheless the time between ribavirin last intake and blood sample collection was not reported in this study. Larrat et al. also demonstrated a relation between SVR and a single-measure

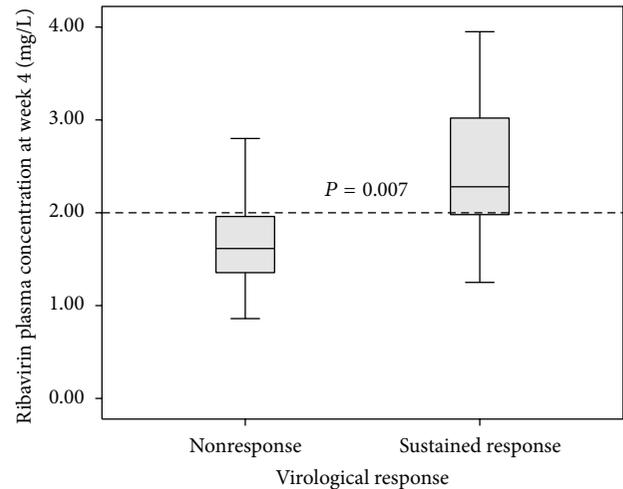


FIGURE 3: Ribavirin plasma concentration at week 4 according to subsequent treatment response. Modified from Maynard et al. [8].

of ribavirin concentration 2–4 hours after the morning intake at W12 of therapy ($n = 24$; $P = 0.03$) [69].

Several studies showed that virological response rate to PEG-IFN alpha-2a/alpha-2b combined with ribavirin increased in relation with ribavirin plasma concentration. Initially Jen et al. showed that virological response rate at W24 increased from 30.9% to 45.2% for an increase of ribavirin concentration from 1.5 mg/L to 3.0 mg/L, respectively [21].

An early measure of AUC at day 0 (D0) was proposed as a predictive value of SVR by Loustaud-Ratti et al. [70]. This prospective observational study of 27 HCV-genotype 1 infected patients showed that AUC_{0-12h} and abbreviated AUC_{0-4h} determined after a first single-dose at D0 was significantly correlated to SVR but also showed that AUC_{0-12h} at W12 was not. Two threshold values were defined for AUC at D0: $3014 \mu\text{g}/\text{h}/\text{L}$ for AUC_{0-12h} with a sensitivity of 91% and a specificity of 61%. For AUC_{0-4h} , the threshold value was $1755 \mu\text{g}/\text{h}/\text{L}$ with a sensitivity of 72% and a specificity of 85%. The assessment of ribavirin AUC_{0-4h} could thus be considered as the most relevant measure due to an easy procedure during ambulatory care of patients and due to a similar predictive value of SVR. For an optimal treatment response, a rapid viral load decrease is necessary during the first weeks or even during the first days of treatment and an early ribavirin dose adjustment thanks to the assessment of ribavirin AUC_{0-4h} at D0 could significantly increase the SVR rate.

In a prospective study of 31 HCV genotype 1 patients, we showed that a ribavirin trough concentration threshold of 2 mg/L at W4 was significantly predictive of SVR [8] (Figure 3). ROC curve analysis showed that this threshold was associated with a sensitivity of 73% and a specificity of 80%. Among patients with a ribavirin concentration >2 mg/L at W4, 67% achieved SVR compared with only 16% if ribavirin concentration was <2 mg/L ($P = 0.007$).

Other studies have evaluated the association between ribavirin concentration and SVR among HCV mono-infected patients. These studies are summarized in Table 1.

TABLE 1: Summary of studies that evaluated the association between ribavirin concentration (CRBV) or AUC and sustained virological response (SVR) rate among HCV monoinfected patients (modified from Solas et al. [89])

Jen et al. [21] 929 naive patients whatever HCV-genotype	CRBV at W4 is a predictive value of SVR. CRBV at W4 >3.5–4 µg/mL is associated with 49% of SVR.
Larrat et al. [69] 24 naive HCV genotype 1 patients	CRBV at W12 and at W24 is significantly higher among patients who achieve SVR compared to null-responders. CRBV at W12 and at W24 >3 µg/mL is associated with SVR.
Tsubota et al. [90] 27 naive HCV genotype 1 patients	CRBV at W4 >2 µg/mL is predictive of SVR.
Maynard et al. [8] 31 HCV genotype 1 patients (8 naive and 23 nonresponder to a previous treatment)	CRBV at W4 >2 µg/mL is predictive of SVR.
Arase et al. [91] 68 naive HCV genotype 1 patients	CRBV at W8 >3 µg/mL is predictive of SVR
Lindahl et al. [78] 10 naive HCV genotype 1 patients	High ribavirin doses were used to achieve a CRRV at W4 >3.6 µg/mL and a CRRV at W12 >3.5 µg/mL in all patients. 9/10 achieved SVR.
Loustaud-Ratti et al. [70] 28 naive HCV genotype 1 patients	AUC_{0–12h} at D0 correlated with AUC_{0–4h} at D0. Both are predictive of SVR. AUC_{0–4h} at D0 > 1755 µg·h/L is predictive of SVR (Se = 72% and Spe = 85%).
Christensen et al. [92]	CRBV at W4 >2 µg/mL is predictive of SVR (PPV = 91%)

8. Relevance of Ribavirin Pharmacologic Monitoring in Specific Therapeutic Situations

8.1. Patients with Renal Insufficiency or on Dialysis. Standard IFN and then PEG-IFN were used during a long time for HCV patients with severe renal insufficiency or on dialysis with dose adjustment, whereas ribavirin was contraindicated in these patients [71]. Few data about potential ribavirin dose adjustment are available since the fear of severe hemolytic anaemia led to low rate of ribavirin prescription in these patients. Bruchfeld et al. were among the first who decided to use ribavirin in such patients. Using ribavirin monitoring, they recommended ribavirin doses adapted to impaired renal function [18–20]. Two pilot-studies with, respectively, 6 and 5 patients reported standard IFN/ribavirin treatment results for dialysis patients. Ribavirin dosage was 600 mg/week for the first study and 200–400 mg/day for the second study with EPO supplementation. These two treatment schedules led to an SVR in 9/11 patients. Treatment was stopped in 3 patients due to side effects [20, 72], two of them because of anemia, and one because of IFN-related side effects. Thereafter, Bruchfeld and others treated dialysis patients with PEG-IFN/ribavirin combination with adjusted ribavirin dosage but not guided by ribavirin monitoring. Deltenre et al. treated 31 patients with PEG-IFN alpha-2a or alpha-2b in combination with ribavirin [73]. Median ribavirin dosage was 112 mg/day and two EPO-strategies were compared (increase of ribavirin dosage considering haemoglobin level or double-dose of EPO before antiviral treatment initiation). Eighteen to 40% of patients received transfusion according to the EPO-strategy and SVR was reached in 48% of cases. More recently, a Taiwanese randomised study compared among dialysis patients a PEG-IFN alpha-2a regimen (135 µg/week) with a PEG-IFN/ribavirin (200 mg/day) combination therapy. Anaemia was more frequent among patients treated by combination therapy but no difference in the rate of treatment

discontinuation was observed between both treatment arms. SVR rate was 64% in the combination therapy arm and 33% in PEG-IFN treated patients ($P < 0.001$) [74]. More recently, a pilot-study showed that four dialysis patients under telaprevir triple therapy had good virological response and tolerance [6].

In this context, we conducted a retrospective study on patients with renal insufficiency, with or without dialysis and treated with IFN (PEG or standard) and ribavirin.

The aim of this study was to evaluate ribavirin efficacy and safety in these patients. The potential association between virological response and ribavirin plasma concentration at W4, W8, and W12 was evaluated. A ribavirin plasma concentration threshold was determined in order to get a reference value for adjustment of ribavirin dosage.

Sixty-three HCV patients (40 males and 23 women) with renal insufficiency were included among whom 42 had moderate renal insufficiency ($30 < \text{creatinine clearance} < 60 \text{ mL/min}$), four had severe renal insufficiency ($15 < \text{creatinine clearance} < 29$), and 17 were on dialysis. Among patients with renal insufficiency 18 had undergone a liver transplantation and 2 patients had a dual kidney/liver transplantation. Thirteen patients had a kidney transplantation and 10 of them needed dialysis during antiviral treatment. Patients with severe or moderate renal insufficiency were pooled due to the small number of patients in each subgroup. Biological parameters, viral load at W4 and W12, and ribavirin plasma concentration at W4, W8, and W12 were assessed. Dosage at the beginning of treatment, dosage adjustment, side effects (in particular haematological side effect), and early treatment cessation were reported.

Patients' characteristics are presented in Table 2. Data on SVR was not available in three patients. Seven patients had an early treatment withdrawal due to side effects: five due to severe anaemia, one due to IFN intolerance, and one patient died from nonhepatic cause. RVR at W4 and at W12 and SVR

TABLE 2: Patient characteristics and initial ribavirin dosage.

Patients <i>N</i> = 63	Dialysis patients (<i>N</i> = 17) (%)	Patients with moderate or severe renal insufficiency (<i>N</i> = 46) (%)
HCV genotype		
1	10 (58.8)	36 (78.3)
2	2 (11.8)	4 (8.7)
3	2 (11.8)	5 (10.5)
4	3 (17.6)	1 (2.3)
Fibrosis		
F0/F1/F2	12 (70.6)	29 (63.0)
F3/F4	5 (29.4)	17 (47.0)
Mean haemoglobin level (g/dL) [IQR]	11.9 [9.9–13.7]	12.7 [11.0–15.1]
Mean ribavirin dosage at D0 (mg/day)	165	560
Mean dosage/weight (mg/kg/day)	2.7	10.4

TABLE 3: Virological response for dialysis patients or for patients with renal insufficiency (perprotocol analysis).

Patients (<i>N</i> = 63)	Dialysis patients (<i>N</i> = 17) (%)	Patients with moderate or severe renal insufficiency (<i>N</i> = 46) (%)
Virological response at W12		
EVR	13/17	23/46
RVR	2/17	1/46
cEVR	7	10
pEVR	4	12
Virological response at W24		
	8/13 (62)	14/40 (35)
HCV genotype		
1	4/8 (50)	8/31 (26)
2	2/2 (100)	3/3 (100)
3	1/1 (100)	3/5 (60)
4	1/2 (50)	0/1 (0)
Fibrosis stage		
F0/F1/F2	6/8 (75)	11/29 (38)
F3/F4	2/5 (40)	6/11 (55)
Early treatment discontinuation		
Severe anaemia	2	5
Death	1	4
Intolerance to interferon	1	0
	0	1

cEVR, complete early virological response; EVR, early virological response; pEVR, partial early virological response; RVR, rapid virological response.

in relation to HCV genotype and liver histology are reported in Table 3.

Among dialysis patients 16/17 received EPO before the beginning of antiviral therapy and all received EPO during therapy. EPO doses were increased in 82.4% of cases. Three patients (17.6%) were transfused. A decrease of ribavirin dosage was proposed for 52.9% of patients. The main reason was a high ribavirin plasma concentration at W4 that could induce severe anaemia. Only one patient stopped treatment because of severe anaemia in a context of orthopedic surgery. In this patient, ribavirin was the probable culprit for the occurrence of anemia.

Among patients with renal insufficiency 39% received EPO and 8.7% (4/46) were transfused (two of them had severe renal insufficiency). A decrease of ribavirin dosage was proposed for 26% of patients and temporary treatment withdrawal for 11%. Antiviral therapy was definitively stopped in four patients (8.7%) due to anaemia despite the use of EPO and/or transfusion. Data on ribavirin plasma concentration at W4, W8, and W12 and treatment response are reported in Table 4. Results indicate that ribavirin plasma concentration was higher in patients with subsequent SVR than in nonresponders or relapsers. Similarly, higher ribavirin levels were observed in patients with rapid virological response (RVR)

TABLE 4: Mean ribavirin plasma concentration.

Patients (N = 63)	Dialysis patients (N = 17)		Moderate or severe renal insufficiency (N = 46)	
	Responder	Nonresponder	Responder	Nonresponder
W4				
EVR	1.47	0.91	2.7	2.36
SVR	1.26 (RR: 0.56)		2.86 (RR: 2.54)	
W8				
EVR	1.48	1.22	2.5	2.15
SVR	1.38 (RR: 0.8)		2.76 (RR: 2.31)	
W12				
EVR	1.65	1.42	2.79	3.07
SVR	1.74 (RR: 1.48)		3.22 (RR: 2.43)	

compared with those failing to achieve RVR, although these differences did not reach statistical significance. In patients with renal insufficiency (excluding dialysis patients) a ROC curve analysis identified a ribavirin concentration threshold of 2.85 mg/L at W4 providing the best sensitivity/specificity (Se 71%, Sp 69%, NPV 85%, and PPV 50%). No association was observed between ribavirin concentration and age, gender, or fibrosis severity.

In this retrospective study and using an intention-to-treat (ITT) strategy, the SVR rate was 53% in dialysis patients and 31% in patients with renal insufficiency. Such results with more favorable outcome in dialysis patients were already reported in studies with IFN alone and were thought to be associated with a better IFN bioavailability. However, results in dialysis patients are not as good as those as the Taiwanese study in which patients were treated with PEG-IFN/ribavirin therapy [74]. This difference is at least partly explained by heterogeneous treatments in our cohort since five patients received standard IFN, 27 PEG-IFN alfa-2a, and 21 PEG-IFN alfa-2b/ribavirin. Several factors could explain the low response rate observed in patients with renal insufficiency. The first one is a poor tolerance in these patients leading to frequent treatment cessation. The second explanation is a high number of patients with liver transplantation in this group, patients for whom SVR is known to be lower and tolerance poorer.

In dialysis patients, treatment was stopped in only one patient because of a severe anemia following orthopedic surgery. In all other cases, no severe anemia was observed probably because of high EPO doses and ribavirin dose adaptation.

In nondialysis patients the number of treatment cessation was also low since only five patients stopped treatment because of intolerance. A severe anemia was the main cause of treatment withdrawal in four of them. In these patients, anemia continued to worsen after the 4th week of treatment and did not respond to EPO which was only introduced after anemia appearance. Ribavirin dose reduction and blood transfusion did not help to adequately control the anemic syndrome. The occurrence of severe anemia in patients with severe renal insufficiency or with a creatinine clearance <40 mL/mn could be explained by Bruchfeld results which

showed that the effect of renal function on ribavirin clearance only appears for a creatinine clearance <34 mL/mn [18]. Noteworthy in these patients, the EPO use is different than in dialysis patients since it is only initiated at the anemia appearance and not before like in dialysis patients. This late EPO use does not avoid the occurrence of severe anemia because of the action delay of this molecule and also because of the increasing accumulation of ribavirin.

In conclusion, it appears that nondialysis patients with severe renal insufficiency are the most difficult to treat. In these patients, ribavirin pharmacological monitoring, dose adaptation, and growth factor use do not allow treatment continuation. If we want to use ribavirin in these patients, it is probably necessary to introduce EPO early like in dialysis patients. In specific cases, it may be preferable to wait the initiation of dialysis before starting a combination therapy. Interestingly, we observed that ribavirin plasma levels were significantly lower in dialysis patients despite a better virological response rate. These results are in contradiction with previous results showing a link between ribavirin exposure and virological response. These better virological results can at least partly be explained by a better efficacy of IFN.

8.2. HIV-HCV Coinfected Patients. As observed in HCV monoinfected patients, there is in HIV-HCV coinfected subjects an interindividual variability of ribavirin plasma concentrations. In these patients, ribavirin exposure is associated with viral efficacy but also with a risk of developing anemia [75]. A possible explanation for lower SVR rate observed in HIV-HCV coinfected patients could be a lower ribavirin bioavailability in these patients. In a study conducted in our center, in 86 HCV patients treated by PEG-IFN/ribavirin, among whom 23 (27%) were HIV coinfected, we assessed ribavirin bioavailability (expressed by AUC_{0-4h}) after an initial ribavirin dose of 600 mg. Blood samples were collected after 30 mn, 1, 2, and 4 hours [7].

Results show that coinfected patients have a significantly lower ribavirin AUC_{0-4h} than monoinfected patients. This ribavirin under-exposure in coinfected patients persisted after normalization of AUC to ribavirin dose per kilogram of body weight and was associated with CD4 level with a

lower AUC in patients with CD4 cell count <500 cells/ μ L. A logistic regression analysis indicated that presence of HIV coinfection and male gender were two independent factors associated with ribavirin under-exposure (defined by AUC <1755 μ g.h/L). However, no association appeared between liver disease severity (fibrosis score) or type of antiretroviral treatment (ART) and AUC. These results suggested a lower ribavirin bioavailability in HIV-HCV coinfecting patients receiving PEG-IFN/ribavirin treatment which could explain the lower SVR rate observed in these patients. Moreover, these results seemed to be associated with the patient immunological status with similar AUC values in mono-infected patients and in coinfecting patients with normal CD4 levels.

Our cohort of HIV-HCV coinfecting patients was representative of the HIV population treated at the hospital (57% with CD4 >500 ; 87% with undetectable viral load under HAART; patients mainly receiving 2 NRTI + 1 boosted protease inhibitor, ref 104). The choice of the AUC_{0-4h} was based on the study by Loustaud-Ratti et al. on HCV mono-infected patients in which the authors showed a very good correlation between AUC_{0-4h} and AUC_{0-12h} [70].

It was previously recommended to use low ribavirin dose in coinfecting patients to avoid toxicity problems associated with other molecules such as zidovudine, stavudine, or didanosine [76, 77]. However, these drugs are progressively neglected to the benefit of less cytotoxic new antiretroviral drugs. Higher ribavirin dose could thus be used in these patients as suggested in other studies [78, 79]. Our results could suggest specific ribavirin dose adjustments in HIV-HCV coinfecting patients, especially according to their immunological status during PEG-IFN based treatment or during new forthcoming treatment strategies.

9. Ribavirin Pharmacokinetics during Triple Therapy

The anemia which is frequently encountered during ribavirin-based treatment of hepatitis C often leads to early treatment modification or withdrawal hereby decreasing the antiviral efficacy. It has been shown that anemia increases during telaprevir exposure [80]. In a study conducted in previous nonresponder HCV patients retreated by telaprevir-based triple therapy, we determined whether telaprevir exposure resulted in increased RIBAVIRIN plasma exposure which could explain the increased incidence of anemia. In parallel, we studied the impact of telaprevir on renal function. Fifty-six HCV patients (among whom 39% cirrhotics) who were nonresponders to a previous IFN/ribavirin therapy and retreated by PEG-IFN/ribavirin/telaprevir were studied.

Ribavirin plasma concentration was measured before telaprevir initiation during the previous course of PEG-IFN/ribavirin combination therapy (T-1), during (at W4 and W8), and after telaprevir treatment (at least 4 weeks after telaprevir withdrawal (W16)).

Ribavirin bioavailability was calculated as the ratio concentration/dosage/body weight.

Our results clearly show a reversible increase of ribavirin bioavailability after telaprevir exposure which might be

linked to the parallel impairment of the estimated glomerular filtration rate (eGFR). It is indeed possible that renal dysfunction due to protease inhibitor increases ribavirin bioavailability hereby inducing anaemia.

10. Discussion

Since more than 15 years, ribavirin plays an important role in HCV treatment. The mechanism of action is complex and remains poorly understood. It is probable that ribavirin acts at different levels of viral replication. Recent studies suggest an action through the IFN system by stimulating IFN genes hereby enhancing the production of endogenous IFN [10]. It has been clearly shown that ribavirin plays a beneficial role on SVR by an action on the second phase of RNA kinetics during combination therapies and on the decreased frequency of relapse after treatment cessation [51]. Early pivotal trials of combination therapy have shown that a minimal ribavirin dose was necessary to achieve SVR. Later on, pharmacological studies have shown that ribavirin trough concentration during the first three months of therapy was correlated with SVR [68, 69]. These studies have defined a threshold predicting response. In parallel, a French team showed that early ribavirin exposure defined by AUC at day 0 was predictive of response [70]. Altogether these results suggested therapeutic strategies based on ribavirin pharmacologic monitoring. However, based on ribavirin pharmacokinetics, the impact on ribavirin exposure only occurs about 4 weeks after dose modification. Therefore, if a ribavirin underexposure is present, it will be observed by ribavirin monitoring only after 4 weeks and the impact of a dose increase will not occur before 8 to 12 weeks which is probably too late. This observation is in favor of rather using the AUC at day 0 to early adapt ribavirin dose.

Another approach consisted in initiating treatment with a ribavirin monotherapy before starting IFN treatment [81-83]. This strategy allowed to introduce IFN when the ribavirin equilibrium was reached, when the ribavirin exposure was satisfactory, and when the action on IFN stimulating genes was effective. However, no study could definitely confirm the efficacy of this strategy in clinical practice. One possible explanation could be the substantial interindividual variability in the ribavirin metabolism. Even if ribavirin pharmacologic monitoring cannot be widely recommended to adapt ribavirin-based treatments, this monitoring may be useful in specific population, especially those with poor ribavirin tolerance. As we previously mentioned it, this is the case in patients with renal insufficiency or patients on dialysis and also in transplanted patients. This is also true in hemolytic pathologies, especially hemoglobinopathies such as thalassemia.

During telaprevir based treatment, the severity of anemia is associated both with toxicity of telaprevir which probably induces a central anemia and with the ribavirin-induced hemolysis [80]. We have shown that ribavirin bioavailability and thus hemolysis were increased after exposure with telaprevir because of a decreased eGFR and also because of a possible pharmacologic interaction (unpublished data). Current recommendations for the management of these

anemia under triple therapy suggest the early reduction of ribavirin dose whether or not HCV RNA negativation has been achieved [84]. We have shown previously that these recommendations were based on studies with small numbers of cirrhotic patients. French recommendations suggest to avoid a too early decrease of ribavirin dose in patients with detectable RNA, especially if they are cirrhotics [85]. In this context, ribavirin pharmacologic monitoring may play a major role in accurately assessing ribavirin bioavailability and in deciding on a potential ribavirin dose adaptation.

Novel shorter and well tolerated therapeutic strategies based on new DAA are very promising with viral eradication in more than 90% of cases. In this context, the role of IFN within these new therapeutic associations will rapidly decrease. However, numerous protocols combining two or three DAA still include ribavirin [86, 87]. Moreover, ribavirin is proposed as a combination therapy with a nucleosidic polymerase inhibitor (sofosbuvir) for the treatment of genotypes 2 and 3 infections [86, 88]. The tolerance of ribavirin seems to be better with this type of association although most treated patients did not have comorbidities or advanced cirrhosis. Results on efficacy are very good in genotype 2 infected patients but are more heterogeneous in case of genotype 3 infection. In this context, ribavirin pharmacologic monitoring could be relevant.

Although the role of ribavirin within new DAA combinations will probably decrease in the future, its potential benefit in difficult-to-treat patients (patients with severe hepatopathy or patients who failed triple therapy including patients with multiresistance) will need to be further investigated.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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