Clinical Infectious Diseases

MAJOR ARTICLE

Pharmacokinetics of Ribavirin in the Treatment of Lassa Fever: An Observational Clinical Study at the Irrua Specialist Teaching Hospital, Edo State, Nigeria

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Background. Lassa fever is endemic in large parts of West Africa. The recommended antiviral treatment is ribavirin. Two treatment regimens are currently endorsed in Nigeria: the "McCormick regimen" based on a study published in 1986 and the "Irrua regimen" constituting a simplified schedule developed at the Irrua Specialist Teaching Hospital, Nigeria. Evidence for the safety and efficacy of ribavirin in Lassa fever patients is poor and pharmacokinetic data for both regimens are lacking

Methods. Polymerase chain reaction-confirmed Lassa fever patients with mild to moderate disease severity were invited to participate in this prospective, observational pharmacokinetic study. Pharmacokinetics of ribavirin, clinical, virologic, and clinical laboratory parameters were assessed.

Results. Using a population pharmacokinetic approach, plasma concentrations of ribavirin were best described by a 3-compartment model. Drug exposure was remarkably consistent between participants. Overall, drug clearance was 28.5% lower in female compared with male participants. Median (5th-95th percentile) time above half maximal inhibitory concentration (IC_{50}) was 37.3% (16.9%–73.1%), 16.7% (8.2%–58.5%), and 9.6% (4.9%–38.4%) on days 1, 7, and 8, respectively. Clinical laboratory parameters indicated reduction of cell damage and development of hemolytic anemia in the course of the treatment period.

Conclusions. This observational study characterizes the pharmacokinetics of ribavirin in the treatment of Lassa fever indicating consistent exposure across patients. Whereas only a short time interval of concentrations above the IC_{50} implies rather low antiviral efficacy in vivo, the prominent reduction of cell damage markers might point to indirect—potentially anti-inflammatory—effects of ribavirin. The role of ribavirin in the treatment of Lassa fever requires further scrutiny.

Keywords. ribavirin; viral hemorrhagic fever; Irrua ribavirin regimen; Lassa fever; Nigeria.

Lassa fever (LF) is a viral hemorrhagic fever endemic in large parts of West Africa and is estimated to cause up to 300 000 infections and more than 5000 deaths per year [1–3]. Transmission from the rodent host to humans follows a seasonal pattern with annual outbreaks during the dry season [4]. Although a high proportion of Lassa virus (LASV) infections are asymptomatic or mild, mortality rates of hospitalized patients may exceed 40% in endemic settings [2, 5, 6]. Because of LF's impact on public health in low- and middle-income countries and the lack of effective medical countermeasures, the World Health Organization has included LF in its Blueprint list of research priorities [7].

Since the 1980s, ribavirin is the standard of care for the treatment of LF. The evidence for this antiviral treatment is largely based on a clinical trial performed in Sierra Leone, published by McCormick et al [8]. Ribavirin therapy as described by McCormick and colleagues consists of intravenous administration of a loading dose of 2 g, followed by 1 g 6 hourly for 4 days, followed by 0.5 g 8 hourly for another 6 days [8]. However, this study and unpublished data from Sierra Leone have been reassessed recently, revealing methodological shortcomings in design, analysis, and reporting of the clinical trial and thus putting in question the evidence base for ribavirin's use in the treatment of LF [9, 10].

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The Irrua Specialist Teaching Hospital (ISTH) in Nigeria is one of the largest LF treatment centers in West Africa, including a laboratory for molecular diagnostics of LF and an isolation ward for up to 50 patients [11]. At ISTH, an easier manageable regimen, the so-called "Irrua ribavirin regimen" was developed, consisting of a 100 mg/kg loading dose on the first day (up to a maximum of 7 g), followed by 25 mg/kg once daily from days 2 to 7 and 12.5 mg/kg once daily from days 8 to 10. Although the Irrua regimen has not been systematically assessed, it became the standard of care at ISTH and one of the nationally recommended treatment regimens for LF in Nigeria [12].

As a first step toward reassessment of ribavirin in the treatment of LF, a prospective observational study was conducted at ISTH with the aim to evaluate the pharmacokinetic properties of the Irrua regimen.

METHODS

Study Design, Setting, Patients, and Treatment

This prospective observational study was conducted in adult nonpregnant LF patients with mild disease course admitted to and routinely managed at ISTH, the protocol was published previously [13]. In brief, adult nonpregnant patients with mild disease progression and real-time reverse transcription polymerase chain reaction (RT-PCR)-confirmed LF infection necessitating routine treatment with intravenous ribavirin were invited to participate. Written informed consent was obtained before any study related procedure. The Irrua ribavirin regimen was routinely administered with individual modifications based on the medical judgment of the treating physicians. The duration of participant follow-up was in line with the duration of routine hospitalization, unless withdrawn early, but did not continue beyond day 11. The study protocol was approved by the Human Research Ethics Committee of ISTH, Nigeria (reference: ISTH/HREC/20190104/009) and the Ethics Committee of the Medical Chamber of Hamburg, Germany (reference: PV7302).

The objectives of this study were published previously [13]. The analysis presented here describes the classical pharmacokinetics (PK) parameters and the evaluation of clinical, hematological, and biochemical parameters in correlation with ribavirin blood levels.

Medical history, previous and concomitant medication, anthropometric measurements and other baseline characteristics, body temperature, signs and symptoms, physical examination, and blood sampling information were collected as part of the study [13]. Participant data were recorded on paper source documents and transcribed to electronic case record forms using REDCap (Version 10.0.25, Vanderbilt, TN, USA). A clinical monitor performed 100% source data verification.

Blood Sampling and Bioanalysis

Venous blood samples for pharmacokinetic analysis were obtained before and 0.5, 1, 3, 5, 8, 12, and 24 hours after routine ribavirin administration on days 1, 4, and 10. Blood samples were immediately centrifuged, and plasma samples were stored within 2 hours after blood collection at −80°C onsite. Subsequently, samples were shipped on dry ice for storage at −80°C in the biosafety level 4 laboratory at BNITM, Hamburg, Germany. Ribavirin was extracted from plasma and LASV was inactivated according to a validated protocol. To this end, plasma was mixed with prechilled (−20°C) acetonitrile in a 1:5 ratio and the mixture was centrifuged at 4°C for 5 minutes at 10 000*g*. Inactivated supernatant was transferred for downstream analysis to the Department of Clinical Pharmacy at the University of Hamburg, Germany. Ribavirin concentration was measured using a validated liquid chromatography coupled to tandem mass spectrometry assay. A brief description of the bioanalytical method is provided in the Supplementary Text and Supplementary Tables 1–3.

Venous blood samples for LASV RT-PCR were taken at screening (day 0), 24 hours after the first routine ribavirin administration (day 2) and then on days 3, 4, 5, 7, 9, and 10. LASV RNA was purified from 70 µL plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and detected using the RealStar Lassa Virus RT-PCR Kit 2.0 (Altona diagnostics) targeting the GPC and the L genes on a Rotor-Gene Q thermocycler (Qiagen) at ISTH.

Venous blood samples for hematology and clinical chemistry were taken on days 0, 3, 5, 7, and 9. Amylase, blood urea nitrogen, creatinine, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), uric acid, and total bilirubin were measured with the SpotChem EZ-SP 4430 analyzer (Axonlab, Baden, Switzerland) at ISTH. Full blood count was measured using the Micros 60 hematology analyzer (Horiba, Kyoto, Japan) at ISTH. Descriptive statistics of baseline characteristics, clinical virology, hematology, and clinical chemistry parameters were done with STATA (Version 16, StataCorp LLC, TX, USA) and Excel (Microsoft, Redmond, WA, USA).

Population Pharmacokinetic Analysis

Ribavirin plasma concentrations were analyzed using nonlinear mixed effects modelling in NONMEM (Version 7.5, ICON, Gaithersburg, MD, USA) using first-order conditional estimation with interaction. Interindividual variability (ie, variability of the pharmacokinetic parameters between patients) and interoccasion variability (ie, variability of the pharmacokinetic parameters across dosing intervals) were modelled assuming a log-normal distribution. One, 2, and 3 compartment models with first-order or Michaelis–Menten elimination were assessed. Model selection was guided by objective function value (a change of the objective function value [dOFV] of −3.84 [ie, $P < .05$] was considered significant for inclusion of 1 additional model parameter), goodness-of-fit plots, and visual predictive checks.

Stepwise covariate modelling ($P < .05$ forward selection and $P < .01$ backward elimination) was used to quantify the impact

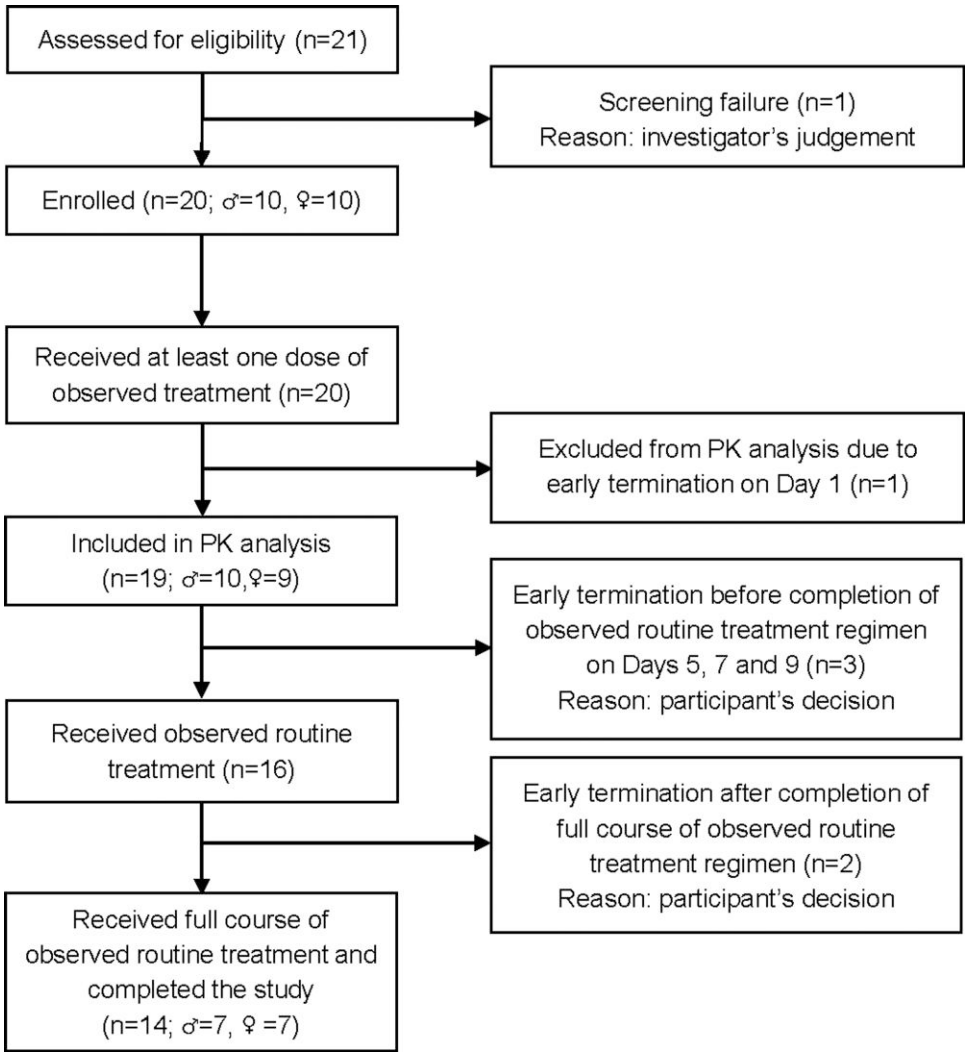

Figure 1. Patient flow.

of patient covariates on pharmacokinetic parameters. Evaluated covariates included body weight (including allometric scaling); markers of renal function (1) serum creatinine, creatinine clearance (estimated by Cockcroft-Gault [14]), (2) Modification of Diet in Renal Disease (MDRD) Formula for Estimation of Glomerular Filtration Rate [15], and (3) Chronic Kidney Disease Epidemiology Collaboration [16]; markers of liver function (AST, ALT, bilirubin, GGT); body height; and sex. In addition to the statistical criteria, selection of covariates for the final model was guided by clinical significance and biological plausibility. Parameter uncertainty was quantified by the log-likelihood profiling-based sampling importance resampling technique [17].

Exploratory graphical analyses were performed to identify potential correlations between ribavirin exposure and uric acid, bilirubin, red blood cell count, and hemoglobin.

Simulations

The time that ribavirin exceeds the half maximal inhibitory concentration (IC_{50}) (26.5 μM [18] [ie, 6.47 mg/L]) or IC_{90} (33.7 μM [18] [ie, 8.23 mg/L]) was assessed using Monte Carlo simulations with the final population pharmacokinetic model and the Irrua regimen as dosing schedule. Therefore, sex-specific body weight was sampled from the mean and variance of the included patient population assuming a log-normal distribution.

Different dosing regimens were investigated to increase the time above IC_{50} to 80% for 95% of the population in an exploratory simulation study.

RESULTS

Participants and Treatment Course

Between February 2020 and March 2021, 21 patients with PCR-confirmed LF were screened and 20 participants were

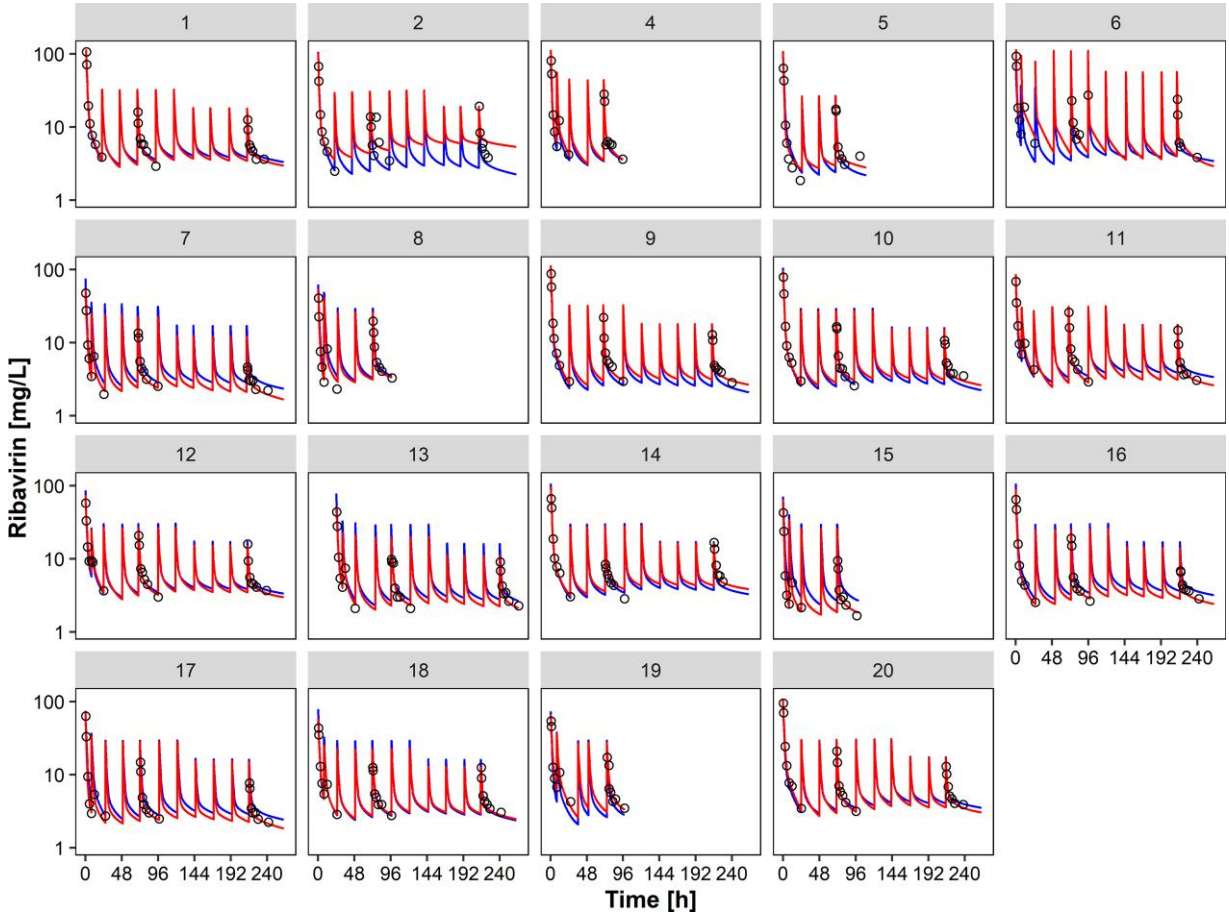

Figure 2. Ribavirin concentration versus time. Red line: individual predicted concentration, blue line: population prediction, circles: measured ribavirin concentration.

enrolled at ISTH. Fourteen participants completed the 10-day study period (Figure 1). Early termination was due to participants' request (n = 5 on days 5, 7, and 9 and 2 on day 10) and because the PCR result from routine laboratory tests could not be confirmed by the research laboratory (n = 1) on the day of inclusion. Nineteen participants were eligible for PK analysis and contributed 381 plasma samples (Figure 2). Two plasma samples were excluded from the pharmacometric analysis because of implausibly high trough concentrations.

Participants' baseline characteristics are depicted in Table 1. Mean age was 33 years and 50% were female. Most frequently reported symptoms during screening were nausea (50%) and intense fatigue and headache (each 25%). Viral load at baseline (day 0) was low with a median cycle threshold (Ct) value of 32.7 and 35.2 for GPC and L gene specific RT-PCR assay, respectively (Table 2 and Supplementary Figure 1). Ct values increased during the study period (Table 2) indicating viral clearance, although 11/19 (58%) participants still had LASV RNA detectable in blood in the last sample taken (Supplementary Figure 1). Important medical events during the observational period were seizures and delirium (n = 1), bacterial infection (n = 1), and co-infections with malaria at baseline (n = 5).

Clinical Chemistry and Hematology

The baseline values and their absolute changes during the study period are shown in Table 2 and Supplementary Figures 2 and 3. Creatinine and blood urea nitrogen levels were in the normal range or marginally elevated during the study period without major changes. Markers of cell damage (AST, ALT, and CPK) were elevated at baseline and tended to decrease or normalize, specifically AST and CPK (median [interquartile range; IQR] of relative change −46% [−70; −32] for AST and −79% [−82; −53] for CPK). Amylase and GGT were normal or elevated but remained stable. Uric acid and bilirubin were within the normal range for most participants at baseline but increased during the study period (median [IQR] of relative change +77% [48; 122] for uric acid and +75% [−6; 137] for bilirubin). White blood cell counts including the fractions of lymphocytes, monocytes, and granulocytes remained stable during the study. Platelets were reduced or

Table 1. Participant Characteristics of Enrolled Patients at Baseline

Parameter		N = 20
Age, y	Median (IQR)	31 (23–43.5)
Female	n (%)	10 (50)
Weight, kg	Mean ± SD	66.9 ± 10.4
Height, cm	Mean ± SD	170.3 ± 8.7
Axillary temperature, °C	Mean ± SD	36.9 ± 0.6
Signs and symptoms		
Nausea	n (%)	10 (50)
Intense fatigue	n (%)	5 (25)
Chest pain	n (%)	4 (20)
Abdominal pain	n (%)	3 (15)
Diarrhea	n (%)	1 (5)
Vomiting	n (%)	1 (5)
Bleeding	n (%)	0 (0)
Hearing problems	n (%)	0 (0)
Decreased vision	n (%)	0 (0)
Other symptoms reported		
Headache	n (%)	5 (25)
Loss of appetite	n (%)	3 (15)
Sore throat	n (%)	3 (15)
Abnormal finding in physical examination:		
Hyperreflexia	n (%)	1 (5)

Abbreviations: IQR, interquartile range; SD, standard deviation.

Table 2. Hematology, Clinical Chemistry, and Virologic Parameters

Parameter	Baseline at Day 0; Median (IQR)	Change During Study Period; Median (IQR)
GPC gene RT-PCR (Ct)	32.7 (28.7; 35.7)	≥5.4 (3.7; ≥9.1)
L gene RT-PCR (Ct)	35.2 (31.1; ≥43.3)	≥4.3 (≥0.2; ≥8.3)
Blood urea nitrogen (mg/dL)	7.0 (6.0; 9.0)	1.0 (0.0; 1.5)
Creatinine (mg/dL)	1.1 (0.9; 1.2)	0.0 (−0.3; 0.2)
ALT (U/L)	48 (26; 103)	−3.0 (−54; 12)
AST (U/L)	67 (54; 200)	−38 (−119; −17)
CPK (U/L)	752 (254; 1074)	−513 (−896; −165)
Amylase (U/L)	104 (90; 132)	8.0 (−2.5; 41)
GGT (U/L)	73 (48; 110)	4.0 (−13; 23)
Bilirubin (mg/dL)	0.8 (0.6; 1.0)	0.4 (−0.1; 0.9)
Uric acid (mg/dL)	2.7 (2.4;4 .0)	2.2 (1.5; 3.2)
White blood cells (10^3/mm^3)	4.7 (3.1; 11.0)	−0.1 (−2.2; 1.1)
Lymphocytes (%)	47 (35; 49)	2.5 (−8.1; 7.3)
Monocytes (%)	8.8 (5.9; 11.5)	0.3 (−3.9; 2.5)
Granulocytes (%)	46 (45; 53)	−1.7 (−9.0; 11.4)
Red blood cells (10^6/mm^3)	4.6 (4.2; 4.7)	−0.6 (−0.8; −0.4)
Hemoglobin (g/dL)	13.3 (12.3; 14.2)	−1.6 (−2.8; −1.5)
Hematocrit (%)	36.8 (35.8; 39.1)	−6.3 (−7.0; −4.6)
Platelets (10^3/mm^3)	207 (104; 345)	126 (−14; 240)

Median and interquartile range (IQR) were calculated for RT-PCR and clinical chemistry for 19 participants and for hematology for 17 participants; hematology values for 2 participants were missing because of lack of reagents while these participants were in the study. The change during study period was calculated for each participant and parameter as difference between value of last sample and value at baseline. A positive and negative difference indicates increase and decrease, respectively, of the values during the study period. Because the Ct value for a negative RT-PCR result is not defined, it was set as Ct ≥ 45 and the minimum change for the lower boundary (Ct = 45) was calculated.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; Ct, cycle threshold; GGT, gamma-glutamyl transferase; RT-PCR, reverse transcription polymerase chain reaction.

within the normal range at baseline and tended to increase in follow-up. A progressive decrease was observed in red blood cell count, hemoglobin, and hematocrit during the study period (median [IQR] of relative change −15% [−17; −10] for red blood cell count, −14% [−20; −11] for hemoglobin, and −16% [−18; −13] for hematocrit). No major differences were seen between men and women (Supplementary Figures 2 and 3).

Population Pharmacokinetics

The population pharmacokinetics of ribavirin was best described by a 3-compartment model with first-order disposition. No saturable elimination (Michaelis–Menten) was detectable. Estimation of interindividual variability (IIV) was supported on clearance (CL), distributional clearance (Q2), and central volume of distribution (V1), whereas the IIV of the remaining parameters tended to zero.

Allometric scaling of all structural model parameters by body weight substantially improved the model fit (dOFV: −19.377) and reduced interindividual variability of CL from 56.3% to 37.34%, whereas the other parameters remained unaffected. Of the other evaluated covariates, sex was significant during forward inclusion (dOFV: −4.325), further reduced interindividual variability on CL from 37.34% to 32.1% and hence was retained in the model. None of the other covariates was significant. The population pharmacokinetic parameter estimates are presented in Table 3. The distribution half-lives were 0.824 ± 0.170 hours and 6.69 ± 1.33 hours, and the terminal half-life was 163 ± 60.9 hours. The individually predicted maximum concentrations reached was 95.1 ± 49.9 mg/L, 32.4 ± 19.4 mg/L, and 19.4 ± 10.9 mg/L on sampling days 1, 4, and 10, respectively (Supplementary Table 4).

Visual predictive checks (Supplementary Figure 4) and goodness of fit plots (Supplementary Figure 5) indicated that the population pharmacokinetic model predicted the observed data well. No significant statistical correlation between ribavirin exposure and the increase or decline of uric acid (P = .227), bilirubin (P = .243), or red blood cell count (P = .663), hemoglobin (P = .965), AST (P = .135), and ALT (P = .686) respectively, were detected (Supplementary Figure 6).

Monte Carlo Simulations

The final population pharmacokinetic model was used for Monte Carlo simulations to compute the time above the IC_{50} and area under the curve (AUC) within 24 hours (Figure 3). The median (5th–95th percentile) for the time above IC_{50} was 37.0% (16.8%–72.4%), 16.5% (8.1%–57.1%), and 9.5% (4.9%–37.1%) on days 1, 7, and 8, respectively (Supplementary Table 5). The results for time above IC_{90} did not exceed 27.7%

Table 3. Typical Pharmacokinetic Parameters (Θ), Unexplained Interindividual Variability (ω_{IIV}) of the Pharmacokinetic Parameters and Residual Variability of Individually Predicted vs Observed Ribavirin Concentrations (σ) for the Final Population Pharmacokinetic Model

Pharmacokinetic Parameter	Estimate	95% CI
Clearance	…	…
$CL = \Theta_{CL} * (1 + COV_{SEX}) * (WT/75)^{0.75}$ [L/h]	…	…
Θ_{CL}	12.0	9.3; 14.9
ω_{IIV} CL	32.1% CV	23.6; 51.3
COV_{SEX} (if female) [–]	−0.284	−0.477; −0.006
Central volume of distribution V1	…	…
$V1 = \Theta_{V1} *$ (body weight/75) [L]	…	…
Θ_{V1}	70.3	56.4; 86.8
ω_{IIV} V1	40.2% CV	27.4; 65.3
Peripheral volume of distribution V2	…	…
$V2 = \Theta_{V2} *$ (WT/75) [L]	…	…
Θ_{V2}	1490	1252; 1799
Peripheral volume of distribution V3	…	…
$V3 = \Theta_{V3} *$ (WT/75) [L]	…	…
Θ_{V3}	113	86.7; 148.4
Distribution clearance for V2	…	…
$Q2 = \Theta_{Q2} * (WT/75)^{0.75}$ [L/h]	…	…
Θ_{Q2}	21.6	17.0; 26.6
ω^2_{IIV} Q2	43.7% CV	31.3; 72.9
Distribution clearance for V3	…	…
$Q3 = \Theta_{Q3} * (WT/75)^{0.75}$ [L/h]	…	…
Θ_{Q3}	20	17.1; 23.0
Residual variability	…	…
$\sigma_{proportional}$	20.8% CV	19.1; 22.9

ω is calculated from the estimated variance of the log-normal distribution $\omega = \sqrt{e^{\omega^2} - 1}$.

(13.7%–53.3%), 10.9% (6.4%–21.2%), and 5.9% (3.0%–11.9%) on days 1, 7, and 8, respectively (Supplementary Figure 7, Supplementary Table 6). Median AUC_{24h} was 242.1 $mgL^{-1}h^{-1}$ (173.9–336.4), 141.3 $mgL^{-1}h^{-1}$ (89.9–193.6), and 113.5 $mgL^{-1}h^{-1}$ (64.3–164.7) on days 1, 7, and 8, respectively. Slightly higher exposure was revealed in female vs male participants (Supplementary Table 7).

Simulations of hypothetical dosing regimens demonstrated that dosing as follows would be required to maintain concentrations around 80% time above the IC_{50} within 24 hours: 2 times 100 mg/kg (limited to 7 g per administration) followed by 3.2- to 6-fold higher daily maintenance doses on day 1, men: day 2, 2× 75 mg/kg; day 3, 2× 50 mg/kg; day 4, 2× 40 mg/kg; days 5–8, 35 mg/kg 2× daily; day 9, 35 mg/kg and 30 mg/kg; day 10, 2×30 mg/kg; women: day 2, 50 mg/kg and 40 mg/kg; day 3, 2× 40 mg/kg; days 4–6, 25 mg/kg 2× daily; day 7, 25 mg/kg and 20 mg/kg; days, 8–10 20 mg/kg 2× daily (Supplementary Figure 8, Supplementary Table 8).

DISCUSSION

Despite being in use for LF treatment since decades, the role of ribavirin and its mode of action in LF patients are still poorly understood. The hypothesis about the mode of action via antiviral activity, however, has been challenged lately [18, 19]. Indeed, when putting our pharmacokinetic findings on time above IC_{50} into perspective, our study suggests that ribavirin may not exert a strong antiviral effect in LF patients treated with the Irrua regimen. A direct evaluation of ribavirin exposure on viral load kinetics was not feasible in this study because of the already low viral load at inclusion and a high proportion of patients with positive RT-PCR at the end of the observational period.

Given the terminal half-life of ribavirin of 163 ± 60.9 h, 815 ± 305 h would be required to reach steady-state conditions. In future studies, extended sampling after the last dose might be of interest to elucidate the distribution volumes of deep compartments and the terminal half-life in more detail. Simulated dosing regimens further indicated that 3.2 to 6 times higher daily doses than those used in the Irrua regimen, together with twice daily dosing, would be needed in more than 95% of the participants' doses to increase the exposure to 80% of time above IC_{50}. Such high doses would be likely correlated with significant toxicity and are unlikely to be feasible in the clinical setting.

Yet, ribavirin may well exert other effects at the currently used dosage, such as immunomodulation. Indeed, experiments in a mouse model and biostatistical modelling suggest that ribavirin reduces cell damage and inflammation by limiting the immune response of the host [19]. This is in line with the strong decrease of cell damage markers, such as AST and CPK in our study. At the same time, ribavirin is known to exert toxic effects in particular on red blood cells and erythropoiesis [20–22]. It is likely that the increase in uric acid and bilirubin, and the decrease in red blood cell count, hemoglobin, and hematocrit are related to this toxicity. However, because this study did not include a control group, it remains speculative whether the alleviation of (inflammatory) cell damage and/or the development of hemolytic anemia are indeed attributable to ribavirin or if they reflect the natural course of LF. Further randomized controlled clinical trials comparing ribavirin to other antiviral drugs or placebo for the treatment of more severe disease with higher viral load will allow to better discern the mode of action and the direct antiviral effect of ribavirin on viral clearance.

Drug exposure was consistent between participants and pharmacokinetic parameters were similarly variable in comparison to other patient populations and indications. For example, similar distribution volumes and distribution clearances of ribavirin were seen in lung transplant recipients; however, ribavirin clearance was found to be substantially higher (12.0 L/h vs 17.5 L/h) [23]. Two further studies in patients with hepatitis C infection also revealed a higher apparent clearance of orally administered ribavirin (oral clearance [CL/F] or 19.0 and 20.5 L/h) that would, however, be close to our determined value if bioavailability of approximately 50% was considered [24, 25].

In this study, body weight, when implemented allometrically, improved the model fit. In addition, sex presented as predictor

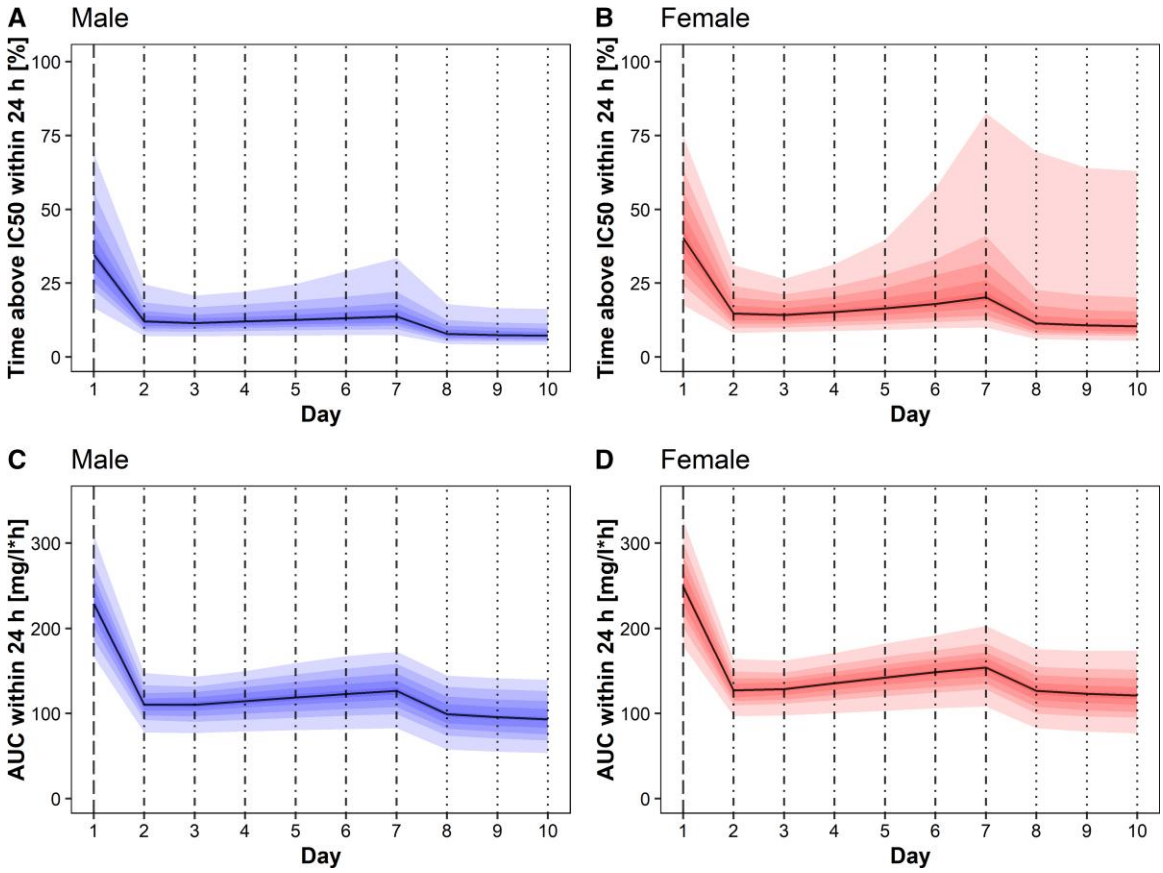

Figure 3. Simulated time above IC_{50} within 24 h for male (*A*) and female population (*B*) and area under the curve (AUC) within 24 hours for male (*C*) and female population (*D*). The population median is given by the solid line and variability is illustrated by shaded areas as 5th to 95th percentile in 10-percentile steps. The vertical lines indicate the changing dosing scheme: dashed line: 100 mg/kg (day 1), dot-dashed line: 25 mg/kg (days 2–7), dotted line: 12.5 mg/kg (days 8–10). IC_{50}, half maximal inhibitory concentration.

for clearance with a 28.4% lower clearance in female participants, independent of the lower body weight in females. Sex was also identified as a covariate on ribavirin clearance previously [26].

A limitation of this study is that the Irrua regimen used in routine care was adapted individually by treating physicians, including a variability in the decrease of dosing to 12.5 mg/kg between days 6 and 8. At the time of protocol development, 100 mg/kg loading dose on the first day of treatment (if total dose >7 g, then 2/3 administered at presentation and 1/3 8 hours later) followed by 25 mg/kg once daily from days 2 to 5 and 12.5 mg/kg once daily from days 6 to 10 were a widely used variation that is reflected in Figure 2 and in the published study protocol [13]. The study team did not interfere with treatment decisions of the treating physician including drug dosage and schedule because of the observational nature of the study design. However, the actual dose and regimen administered, and accurately documented dosing and sampling times were used for the pharmacokinetic analysis thus avoiding potential confounding by altered drug dosage. The study protocol dosing regimen was used for the prediction of IC_{50} and AUC [13].

In summary, results of this observational pharmacokinetic study support the hypothesis that ribavirin acts through a mode of action other than antiviral. This understanding puts into question the rationale for the high ribavirin doses recommended for LF treatment that are associated with important toxicity. Further research is required to refine the role of ribavirin in LF treatment as well as to identify alternative treatment candidates with proven antiviral activity in vivo.

Supplementary material

Supplementary material is available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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F. O. B., J. H., J. N., J. M., M. H., M. P., M. G., A. T., L. O., and S.D. Data interpretation: C. J. K., S. G. W., S. G., M. I. R., and M.G. All authors contributed to the development of the paper, provided critical review, and approved the final version for submission. All authors accept responsibility for the content of this paper.

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