


ARTICLE

A randomized, placebo-controlled study to evaluate safety and pharmacokinetics of inhaled ribavirin

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Funding information

The study was funded by Bausch Health Inc. Research and Development division. The study was designed and supported by Cliantha Research, Mississauga, Canada.

Abstract

Ribavirin is an inosine monophosphate dehydrogenase inhibitor. Studies suggest ribavirin aerosol could be a safe and efficacious treatment option in the fight against coronaviruses. However, current treatment is long (12–18 h per day, 3–7 days), limiting clinical utility. A reduction in treatment time would reduce treatment burden. We aimed to evaluate safety and pharmacokinetics (PK) of four, single-dose regimens of ribavirin aerosol in healthy volunteers. Thirty-two subjects were randomized, to four cohorts of aerosolized ribavirin (active) or placebo. Cohort 1 received 50 mg/ml ribavirin/placebo (10 ml total volume); cohort 2, 50 mg/ml ribavirin/placebo (20 ml total volume); cohort 3, 100 mg/ml ribavirin/placebo (10 ml total volume); and cohort 4, 100 mg/ml ribavirin/placebo (20 ml total volume). Intense safety monitoring and PK sampling took place on days 1, 2, 3, and 40. Subjects were (mean \pm SD, active vs. placebo) aged 57 ± 4.5 vs. 60 ± 2.5 years; 83% vs. 88% were female; and 75% vs. 50% were Caucasian. Some 12.5% (3/24) and 25% (2/8) experienced at least one treatment-emergent adverse event (TEAE) (two moderate; five mild) in the active and placebo groups, respectively. No clinically significant safety concerns were reported. Mean maximum observed concentration (C_{max}) and area under the curve (AUC) values were higher in cohort 4, whereas cohorts 2 and 3 showed similar PK values. Ribavirin absorption reached C_{max} within 2 h across cohorts. Four single-dose regimens of ribavirin aerosol demonstrated systemic exposure with minimal systemic effects. Results support continued clinical development of ribavirin aerosol as a treatment option in patients with coronaviruses.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Ribavirin is an inosine monophosphate dehydrogenase inhibitor, an enzyme in the synthesis of purine nucleotides, and a broad-spectrum antiviral agent. It is approved in the USA and Canada for the treatment of lower respiratory tract infections in hospitalized infants and children due to the respiratory syncytial virus

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(RSV). Early data suggest that ribavirin is safe and effective in the treatment of COVID-19. However, RSV treatment procedures are lengthy (12–18 h per day for 3–7 days), limiting wider clinical utility. A shorter treatment time, while maintaining safety and efficacy, is required for ribavirin to become a practical treatment option for coronaviruses.

WHAT QUESTION DID THIS STUDY ADDRESS?

We performed this study to evaluate the safety and pharmacokinetics (PK) of four, single-dose, clinically relevant regimens of ribavirin aerosol in healthy volunteers. Doses ranged from 50 to 100 mg/ml, delivered in a single inhalation of either 20 or 40 min duration.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Our results showed that four single-dose regimens of ribavirin aerosol were safe and well-tolerated, without dose-limiting toxicities, and a comparable safety profile to placebo. The PK were linear and well-tolerated across the four single-dose regimens, demonstrating systemic exposure with minimal systemic effects.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

In the context of coronaviruses, delivery of drug directly to the site of infection (the respiratory tract) is key. As such, these results support the continued clinical development of ribavirin aerosol as a new treatment option in patients with coronaviruses.

INTRODUCTION

In the last decade, discoveries of new viruses have increased exponentially, largely due to advances in research technology.¹ Since the start of the coronavirus pandemic, the world has been focusing on coronavirus 2 (SARS-CoV-2), as it has led to “dramatic loss of human life worldwide.”² SARS-CoV-2 has been associated with excessive proinflammatory responses in some patients, which can lead to acute respiratory distress syndrome, pulmonary ground glass opacities, severe lung pathology, and death.^{3,4}

Ribavirin is an inosine monophosphate dehydrogenase inhibitor, an enzyme in the synthesis of purine nucleotides.⁵ It is currently approved in the USA and Canada for the treatment of lower respiratory tract infections in hospitalized infants and children due to the respiratory syncytial virus (RSV). Ribavirin has *in vitro* activity against many highly lethal emerging viruses and is considered a broad-spectrum antiviral agent.⁶ It has been shown to inhibit ribonucleic acid (RNA) synthesis by interrupting activity of viral RNA-dependent RNA polymerases, suggesting it could be effective against coronaviruses.⁷

Ribavirin shows rapid absorption and distribution phases and a long terminal clearance phase.⁸ The volume of distribution is high (several 1000 L). Transport to non-plasma compartments has been mainly studied in red cells, and the ratio of distribution among whole blood and plasma is 60:1.⁸ Red blood cells (RBCs) have the capacity to phosphorylate ribavirin to ribavirin monophosphate,

ribavirin diphosphate, and ribavirin triphosphate, but they are devoid of phosphatase activity to convert them back to ribavirin.⁸ As a result, high levels of phosphorylated ribavirin accumulate intracellularly. Since the phosphorylated metabolites are mostly partitioned intracellularly, total ribavirin was assessed in whole blood in addition to ribavirin in plasma to provide a more complete representation of ribavirin’s pharmacokinetic profile. Determination of ribavirin in samples was analyzed using a validated liquid chromatography–tandem mass spectrometry method.^{9–11}

Ribavirin is formulated for use via inhalation, intravenous administration, or oral route making it accessible across clinical scenarios. Studies have reported ribavirin aerosol to be active and well-tolerated against specific influenza strains.^{12–14} In addition, a recent phase II, randomized trial conducted in China found that triple therapy (interferon beta-1b, lopinavir-ritonavir, and oral ribavirin) was safe and effective in alleviating symptoms and shortening the duration of viral shedding and hospital stays in patients with mild to moderate COVID-19.³ Ribavirin aerosol was recently administered at 100 mg/ml for 30 min twice daily for 6 days, in Italy for compassionate use in COVID-19 patients experiencing respiratory distress.¹⁵ Results showed that patients fully recovered with negative nasopharyngeal swabs upon hospital discharge and no reported adverse events (AEs) associated with ribavirin treatment. Together, these data suggest that ribavirin aerosol could be a safe and efficacious treatment option in the fight against coronaviruses. However, the current approved use of ribavirin aerosol for RSV is

20 mg/ml, administered continually for 12–18 h per day for 3–7 days.¹⁶ Such long treatment limits clinical utility and patient care in the COVID-19 setting. Using animal models, Gilbert and McLeay¹⁷ demonstrated that by increasing ribavirin concentration in the reservoir, the time of treatment could be significantly reduced to a single daily 30 min exposure. This reduction in treatment time, with the ability to maintain safety and efficacy, would suggest ribavirin could be a practical treatment option in the fight against coronaviruses at a time when the world is experiencing emerging variants of SARS-CoV-2 and multiple lines of therapies are key. We conducted a dose study in healthy volunteers with the primary objective being to evaluate the safety and pharmacokinetics (PK) of four single-dose, clinically relevant regimens of ribavirin, administered via a high-efficiency air-jet nebulizer.

METHODS

Subjects

Healthy male and female volunteers were enrolled in the study. To be eligible to participate in the study subjects were: adult male or female ≥ 18 and ≤ 65 years of age; females must have been of non-childbearing potential at least 6 months prior to screening [hysteroscopic sterilization, bilateral tubal ligation or bilateral salpingectomy, post-menopausal with spontaneous amenorrhea for ≥ 12 consecutive months with follicle-stimulating hormone > 25.8 mIU/ml; or having had bilateral oophorectomy (with or without hysterectomy)]; males must have had a vasectomy with confirmed post-vasectomy semen analysis or with a documented diagnosis of infertility; body mass index (BMI) ≥ 18.50 and < 30.00 kg/m²; in good general health without clinically significant hematological, cardiac, respiratory, renal, endocrine, gastrointestinal, psychiatric, hepatic, or malignant disease; and suitable venous access for blood sampling. Exclusion criteria were: hypersensitivity to ribavirin; asthma, chronic obstructive pulmonary disease (COPD), or bronchospasm; anemia; any major illness or systemic infection (including COVID-19) within 4 weeks of screening or a clinically relevant history or suffering from a disease or condition that may affect the evaluation of the study drug or place the subjects at undue risk; seropositive for human immunodeficiency virus, hepatitis C virus, hepatitis B virus, or positive for SARS-CoV-2 at screening; had a clinically relevant history of or current evidence of abuse of alcohol or other drug(s); was currently a tobacco smoker or was a tobacco smoker within 6 months of the baseline visit; had donated 500 ml or more of blood in the previous 56 days; was currently participating in another clinical study.

Subjects were asked to refrain from taking any prescription or non-prescription medications up to 14 days prior to the first dose of study drug through to study end. If it was necessary to treat a minor ailment, ibuprofen (maximum 400 mg/day and up to 1200 mg/week) and acetaminophen (maximum 1000 mg/day) were permitted. No concomitant procedures (e.g., surgery/biopsy) or diagnostic assessment (e.g., blood gas measurement) were permitted.

The study was conducted according to the guidelines set out by the International Council for Harmonisation Guidelines for Good Clinical Practice, the ethical principles outlined in the Tri-Council Policy Statement and Canadian regulatory requirements. All subjects gave written informed consent prior to participating. The study was registered on www.clinicaltrials.gov (NCT05229510).

Study design

This was a phase Ia, randomized, double-blind, placebo-controlled, safety, PK, single-ascending dose study. Subjects were randomly assigned to active or placebo group in a 6:2 ratio. Four successive cohorts of eight subjects were enrolled, each cohort receiving a single dose of ribavirin or placebo using different delivery regimens (for full details on dosing and how ribavirin and placebo were prepared and administered refer to the [Online Supplement](#)). Cohorts were,

- Cohort 1: 50 mg/ml ribavirin or placebo (10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min).
- Cohort 2: 50 mg/ml ribavirin or placebo (20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min).
- Cohort 3: 100 mg/ml ribavirin or placebo (10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min).
- Cohort 4: 100 mg/ml ribavirin or placebo (20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min).

Screening (day –14 to day –1)

Subjects were screened for eligibility within 14 days prior to enrollment to confirm inclusion criteria and prepare for dosing on day 1. During screening, the following data were collected; demographics, medical history, current medications, BMI, vital signs, a complete physical examination, 12-lead electrocardiogram (ECG), blood samples for hematology, chemistry, and virology, urine sample for urinalysis, and spirometry. All eligible subjects were

scheduled for a baseline visit within 14 days of screening. Subjects were asked to be mindful of social distancing practices to ensure a COVID infection was not contracted. If the subject required a washout period, it was completed prior to the baseline visit. Subjects were admitted to the clinical site for an overnight stay prior (day -1) to baseline. Subjects began fasting a minimum of 10 h prior to the study drug administration and continued fasting through 4 h after completion of study drug administration on day 1. Upon admission to the clinical site, all subjects had a urine drug screen and alcohol test to confirm eligibility. Subjects underwent SARS-CoV-2 testing at this time via nasal/nasopharyngeal/oropharyngeal swab.

Baseline (day 1)

The following assessments were performed; all medical procedures, changes in concomitant medications and AEs were recorded; a pre-dose spirometry test within 1 h prior to study drug administration; physical examination; blood samples for hematology and chemistry; urine sample for urinalysis; vital signs within 1 h prior to study drug administration. If the subject remained eligible, they were randomized to a treatment cohort and a pre-dose PK sample was taken. Treatment was administered as per assigned cohort (Figure 1). Vital signs were recorded after study drug administration (30 min) and before collection of first post-dose PK sample. PK samples were obtained at 10, 20, 40, 60, 90, 120, and 150 min and 3, 4, 8, and 12 h after study drug administration. For cohorts 1 and 3 (Figure 1), the 20 min sample collection corresponded with the solution/fluid depletion. For cohorts 2 and 4 (Figure 1), the 40 min time point corresponded with the solution/fluid depletion. An ECG was obtained 1 h after completion of study drug administration.

Day 2

Subjects remained overnight on day 1 and were discharged from the site on the morning of day 2 after completion of the following procedures: all changes in concomitant medications and AEs were recorded; vital signs were obtained prior to collection of 24 h PK sample; PK sample 24 h after the start of study drug administration on day 1.

Day 3

On day 3, the same assessments were completed as on day 2. In addition, a 48 h PK sample was collected, along with blood samples for hematology and chemistry and urine

sample for urinalysis. The end of study (EOS) visit was scheduled.

Day 40

The same assessments were completed as on day 3. In addition, a 40-day PK sample was collected. Subjects were discharged from the study.

Bioanalytical method

Determination of ribavirin (free) and total ribavirin in plasma and whole blood samples were analyzed using a validated liquid chromatography–tandem mass spectrometry method.^{9–11} For more details on the bioanalytical methods refer to the [Online Supplement](#).

Safety analysis

All AEs from the time of informed consent until EOS were recorded for seriousness, severity, and relationship to study drug treatment. A respiratory therapist was present with each subject for the entire duration of treatment. During this time all AEs were monitored and reported to ensure subject safety. In addition, a safety monitoring committee addressed safety throughout the trial and ensured all AEs were reported. The full schedule of assessments (Table S1) and clinical/laboratory tests performed (Table S2) are shown in the [Online Supplement](#).

Statistical analysis

No sample size estimation was conducted. The sample size of eight subjects per treatment cohort receiving ribavirin (active group) and two subjects receiving placebo (placebo group) was believed to be adequate to meet the objectives of this study based on previous PK phase I experience.

Safety was the primary outcome measure and assessed by the proportion of subjects with treatment-emergent adverse events (TEAEs), changes in vital signs, physical examinations, laboratory tests, ECGs, and spirometry. The safety population was defined as all randomized subjects that received the study drug and were analyzed based on the treatment received, regardless of the cohort to which they were assigned. All AEs were classified using MedDRA terminology. Descriptions of TEAEs included the date of onset, the date the AE ended, serious (Y/N), the severity of the

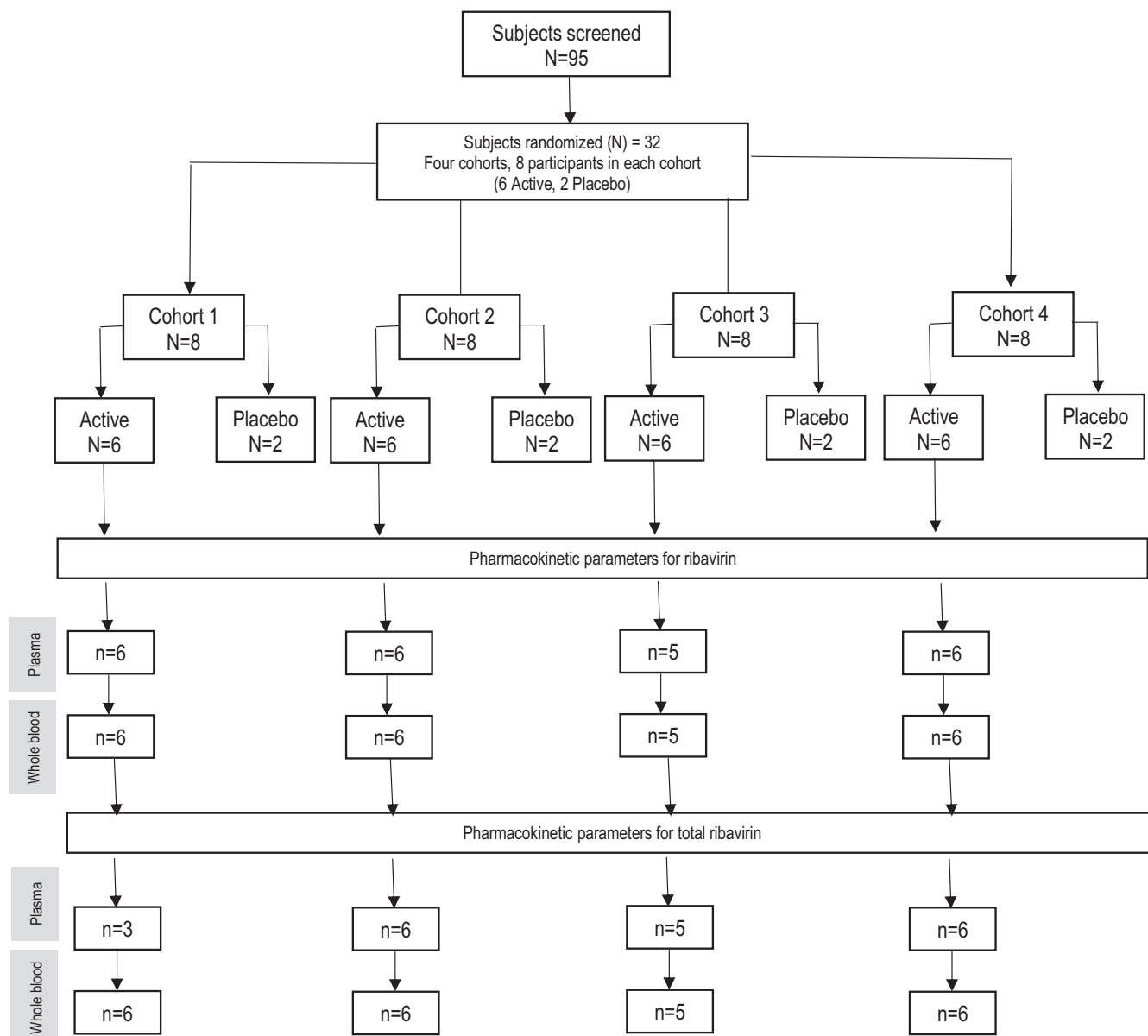


FIGURE 1 Subject flowchart. Cohort 1: 50 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min). Cohort 2: 50 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min). Cohort 3: 100 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min). Cohort 4: 100 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min). N , number of subjects.

AE (mild, moderate, severe), the relationship to study drug, the action taken regarding study drug usage, the action taken to treat the AE, and the outcome. TEAEs were summarized for the entire population, by cohort, and by treatment. Each subject was counted only once by using the AE with the highest severity or greatest relationship, respectively, within each category. Changes from baseline in safety laboratory values and vital sign measurements were summarized with descriptive statistics for each cohort and treatment at all applicable study

visits. Subjects were assessed for dose-limiting toxicities (DLTs) to determine if the highest tolerated dose for any cohort had been reached. Refer to the [Online Supplement](#) for DLT definitions.

The PK population was defined as all subjects that received the study drug and had at least one evaluable blood sample collected and analyzed for plasma and/or whole blood levels. The PK parameters were calculated using WinNonlin® professional software (Phoenix® version: 8.1 Pharsight Corporation), and derived using a

non-compartmental approach. Maximum observed concentration (C_{\max}) time to reach C_{\max} (T_{\max}) and area under the curve to the last measurable concentration (AUC_{last}), were recorded. The PK sampling schedule did not allow for full assessment of half-life and as such these data are not reported. If a subject missed a scheduled visit, the visit was considered missed. Subjects who did not return for the day 3 or 40 visits and could not be contacted, were considered lost to follow-up. There was no imputation done for any missing samples. For continuous variables, the mean, standard deviation (SD), median, and range are presented. Categorical variables are summarized by proportions. All statistical procedures were completed using SAS software (version 9.4; SAS Institute).

RESULTS

Demographics

A total of 95 subjects were screened and 32 were randomized and completed the study (Figure 1). Sixty-three patients did not meet the inclusion criteria. Demographic data for the safety population are shown in Table 1. The mean age was 57 years in the active and 60 years in the placebo groups. Overall, there was a greater percentage of females than males in the study (83% vs. 16% active group; 87% vs. 13% in placebo group, respectively). Most subjects were Caucasian (75% in active and 50% in placebo group). Average BMI was similar in both active and placebo groups (26.1 vs. 26.3 kg/m², respectively). All baseline parameters were similar across the cohorts in both active and placebo groups. No clinically significant abnormalities were reported in any of the baseline characteristics in active or placebo groups.

Safety

All subjects received all assigned doses. Overall, 12.5% (3/24) of subjects dosed with ribavirin and 25% (2/8) of subjects in the placebo group experienced at least one TEAE (Table 2). These TEAEs were mild/moderate in severity with no subjects discontinuing or withdrawing from treatment. In cohorts 1 and 4 (active group, Figure 1), there was 1 moderate AE (headache) reported by at least 1 subject. Several AEs (ventricular extrasystoles, increased aspartate aminotransferase, increased systolic blood pressure, nausea, and vomiting) which were mild in severity were reported in cohort 1 (placebo group, Figure 1) and cohort 4 (active group, Figure 1). The majority of TEAEs were unrelated to study treatment and were resolved without taking any action. Only one subject had

to take concomitant medication to resolve a headache. No serious or severe TEAEs were reported in any cohort. There were no clinically meaningful treatment or dose-dependent differences in the mean change from baseline values for hematology parameters between the active and placebo groups in the study. One subject from the active group of cohort 4 (Figure 1) showed higher creatine kinase pre-dose (day 1) and post-dose (day 3) and higher aspartate aminotransferase (day 3), which were clinically significant. Otherwise, there were no clinically meaningful treatment or dose-dependent differences in the mean change from baseline values for the chemistry between the active and placebo groups. There were no clinically meaningful treatment or dose-dependent differences in the mean change from baseline values for urinalysis, chemical, or microscopy parameters between the active and placebo groups. One subject in the placebo group of cohort 1 (Figure 1), on day 1, showed increased systolic blood pressure which was reported as a TEAE. No other subjects reported any vital sign TEAEs. There were no clinically meaningful differences in ECGs between the active and placebo groups. All ECG results were in the normal range except one subject in the placebo group from cohort 1, on day 1, who showed an abnormal, clinically significant ECG result (sinus bradycardia with frequent ventricular premature complexes in bigeminal pattern and sinus rhythm with frequent ventricular premature complexes in a bigeminal pattern). There were no clinically meaningful differences in the spirometry parameters during the screening, pre-, and post-dose in any group. Lastly, there were no clinically meaningful differences in the physical examination during the screening period between the active and placebo groups.

Pharmacokinetic evaluations

The plasma and whole blood samples of 32 subjects were assayed for ribavirin and total ribavirin. One subject from the active group (cohort 3, Figure 1) showed below the lower limit of quantification (LLOQ) concentrations for ribavirin and total ribavirin in plasma and whole blood. In addition, total ribavirin in plasma for three subjects (cohort 1, Figure 1) were not considered for PK analysis since the profile did not include a minimum of four consecutive plasma concentrations. The later observations reflect that the bioanalytical method presents a 10-fold higher LLOQ for total ribavirin (0.254 µg/ml) compared to ribavirin (0.02 µg/ml) leading to additional below the level of quantification (BLQ) data for total ribavirin in plasma.

All subjects' mean plasma ribavirin and total ribavirin concentration profiles over time, after ascending dose, are shown in Figure 2 for the active group. Ribavirin

TABLE 1 Demographic characteristics of the safety population

Characteristic	Cohort 1		Cohort 2		Cohort 3		Cohort 4		Total	
	Active (N = 6)	PL (N = 2)	Active (N = 6)	PL (N = 2)	Active (N = 6)	PL (N = 2)	Active (N = 6)	PL (N = 2)	Active (N = 24)	PL (N = 8)
Age (years)										
Mean (SD)	56.8 (4.8)	60.5 (0.7)	57.2 (6.7)	61.0 (4.2)	57.8 (3.5)	57.0 (1.4)	57.0 (3.4)	61.5 (0.7)	57.2 (4.5)	60.0 (2.5)
Sex, n (%)										
Male	1 (17)	1 (50)	1 (17)	0	1 (17)	0	1 (17)	0	4 (17)	1 (13)
Female	5 (83)	1 (50)	5 (83)	2 (100)	5 (8)	2 (100)	5 (83)	2 (100)	20 (83)	7 (87)
Race, n (%)										
Caucasian	5 (83)	1 (50)	6 (100)	1 (50)	3 (50)	0	4 (67)	2 (100)	18 (75)	4 (50)
Asian	1 (17)	1 (50)	0	0	1 (17)	1 (50)	1 (17)	0	3 (13)	2 (25)
Black or African American	0	0	0	1 (50)	2 (33)	1 (50)	1 (17)	0	3 (13)	2 (25)
Height (cm)										
Mean (SD)	162.9 (6.5)	161.3 (21.0)	164.7 (7.0)	157.2 (11.2)	160.6 (6.0)	158.2 (7.5)	166.4 (8.3)	160.9 (2.1)	163.7 (6.9)	159.4 (9.6)
Weight (kg)										
Mean (SD)	69.9 (11.1)	74.8 (17.9)	68.6 (9.8)	68.4 (14.5)	66.6 (10.7)	61.1 (8.9)	76.8 (12.0)	64.4 (3.3)	70.4 (11.2)	67.2 (10.9)
BMI (kg/m ²)										
Mean (SD)	26.2 (2.6)	28.6 (0.5)	25.3 (3.3)	27.5 (1.9)	25.7 (2.9)	24.3 (1.2)	27.6 (2.5)	24.9 (1.9)	26.2 (2.8)	26.3 (2.2)

Note: All data are presented as mean \pm SD, unless stated otherwise. Cohort 1: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 2: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 3: 100 mg/ml ribavirin (active) or placebo aerosolized. Cohort 4: 100 mg/ml ribavirin (active) or placebo aerosolized.

Abbreviations: BMI, body mass index; N, number of subjects in the specified group; PL, placebo; SD, standard deviation.

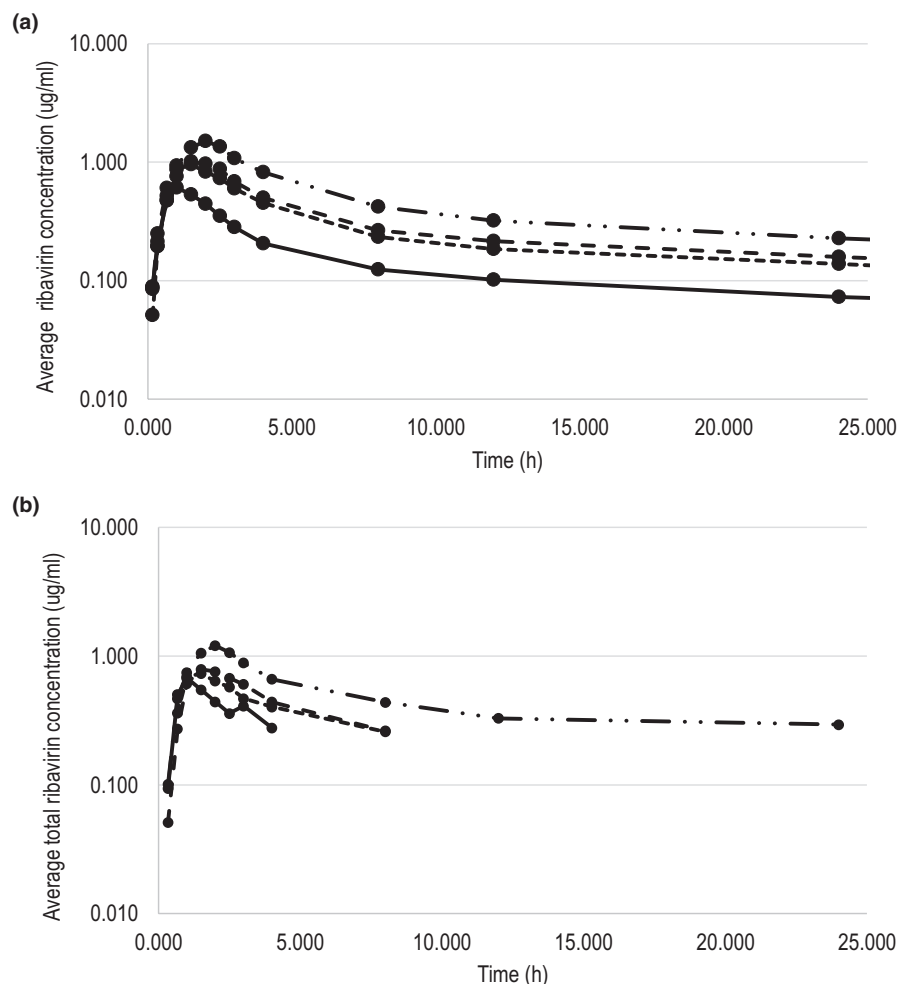
TABLE 2 Summary of treatment-emergent adverse events by cohort

TEAE	Cohort 1		Cohort 2		Cohort 3		Cohort 4		Total	
	Active (N = 6)	PL (N = 2)	Active (N = 6)	PL (N = 2)	Active (N = 6)	PL (N = 2)	Active (N = 6)	PL (N = 2)	Active (N = 24)	PL (N = 8)
Overall TEAEs										
Subjects with TEAEs, <i>n</i> (%)	1 (16.6)	2 (100)	0	0	0	0	2 (33.3)	0	3 (12.5)	2 (25.0)
Events, <i>n</i>	1	2	0	0	0	0	4	0	5	2
TEAEs by category										
Ventricular extrasystoles	0	1 (50.0)	0	0	0	0	0	0	0	1 (12.5)
Nausea	0	0	0	0	0	0	1 (16.7)	0	1 (4.2)	0
Vomiting	0	0	0	0	0	0	1 (16.6)	0	1 (4.1)	0
Aspartate aminotransferase increase	0	0	0	0	0	0	1 (16.6)	0	1 (4.1)	0
Blood pressure systolic increase	0	1 (50.0)	0	0	0	0	0	0	0	1 (12.5)
Headache	1 (16.6)	0	0	0	0	0	1 (16.6)	0	2 (8.3)	0

Note: Percentages are calculated based on the number of subjects in the specified treatment group and overall. Multiple reports of the same TEAE for the same subject were counted once per category. Medical Dictionary used: MedDRA Version 24.0. Cohort 1: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 2: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 3: 100 mg/ml ribavirin (active) or placebo aerosolized. Cohort 4: 100 mg/ml ribavirin (active) or placebo aerosolized.

Abbreviations: N, number of subjects in the specified cohort/treatment group; *n*, number of subjects reporting at least one incidence of the specified adverse event (AE); PL, placebo; TEAE, treatment-emergent adverse event.

FIGURE 2 Semi-log plot of plasma concentration–time profiles of (a) ribavirin and (b) total ribavirin after ascending doses of ribavirin aerosol up to 48 h post-dose in the active groups. Cohort 1: 50 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min). Cohort 2: 50 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min). Cohort 3: 100 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min). Cohort 4: 100 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min). N , number of subjects.



absorption after inhalation reached maximum concentration within 2 h in all the cohorts. All subjects' mean whole blood ribavirin and total ribavirin concentration over time, after ascending dose, are shown in Figure 3 for the active group. At day 40, ribavirin plasma and whole blood concentrations, as well as whole blood total ribavirin, were BLQ.

Table 3 provides a summary of PK parameters for plasma ribavirin. The maximum concentration of ribavirin in cohort 4 (20 ml of 100 mg/ml) was 1.64 $\mu\text{g/ml}$ (plasma) and 1.46 $\mu\text{g/ml}$ (whole blood). Mean C_{max} and AUC_{last} plasma values were highest in cohort 4 where subjects received 20 ml of ribavirin 100 mg/ml. Cohort 2 and 3 (20 ml of 50 mg/ml and 10 ml of 100 mg/ml) showed similar PK plasma values. Cohort 4 had 2–3-fold higher mean C_{max} and AUC_{last} compared with cohort 1 (10 ml of 50 mg/ml). Mean C_{max} and AUC_{last} whole blood values were highest in cohort 4, with cohorts 2 and 3 showing similar PK whole blood values. Cohort 4 had 2–3-fold higher PK values compared to cohort 1. Calculations for the placebo PK parameters could not be made since the ribavirin was BLQ. Table 4 provides a summary of PK parameters for total ribavirin in all subjects dosed with ribavirin. The maximum concentration of total ribavirin in cohort 4 was

1.32 $\mu\text{g/ml}$ (in plasma) and 6.19 $\mu\text{g/ml}$ (in whole blood). Mean C_{max} and AUC_{last} plasma values were highest in cohort 4. Cohorts 2 and 3 showed similar PK plasma values. Cohort 4 had 2–5-fold higher mean C_{max} and AUC_{last} compared with cohort 1. Mean C_{max} and AUC_{last} whole blood values were highest in cohort 4. Cohorts 2 and 3 showed similar PK whole blood values. Cohort 4 was 3–5-fold higher in mean C_{max} and AUC_{last} compared to cohort 1. Calculations for the placebo PK parameters could not be made since the ribavirin was BLQ.

DISCUSSION

At a time when the world is experiencing emerging variants of SARS-CoV-2, multiple lines of therapies are key to help address the global pandemic. Our study provides reassuring data showing that ribavirin aerosol can be administered for short durations at a standard dose safely and does not produce any of the side effects previously associated with ribavirin treatments.^{8,12–14,18} Ribavirin IV/oral have been associated with mutagenicity,^{19–21} teratogenicity in women of childbearing age,²² and anemia²³; while reticulocytosis,²⁴ conjunctivitis,²⁵ and

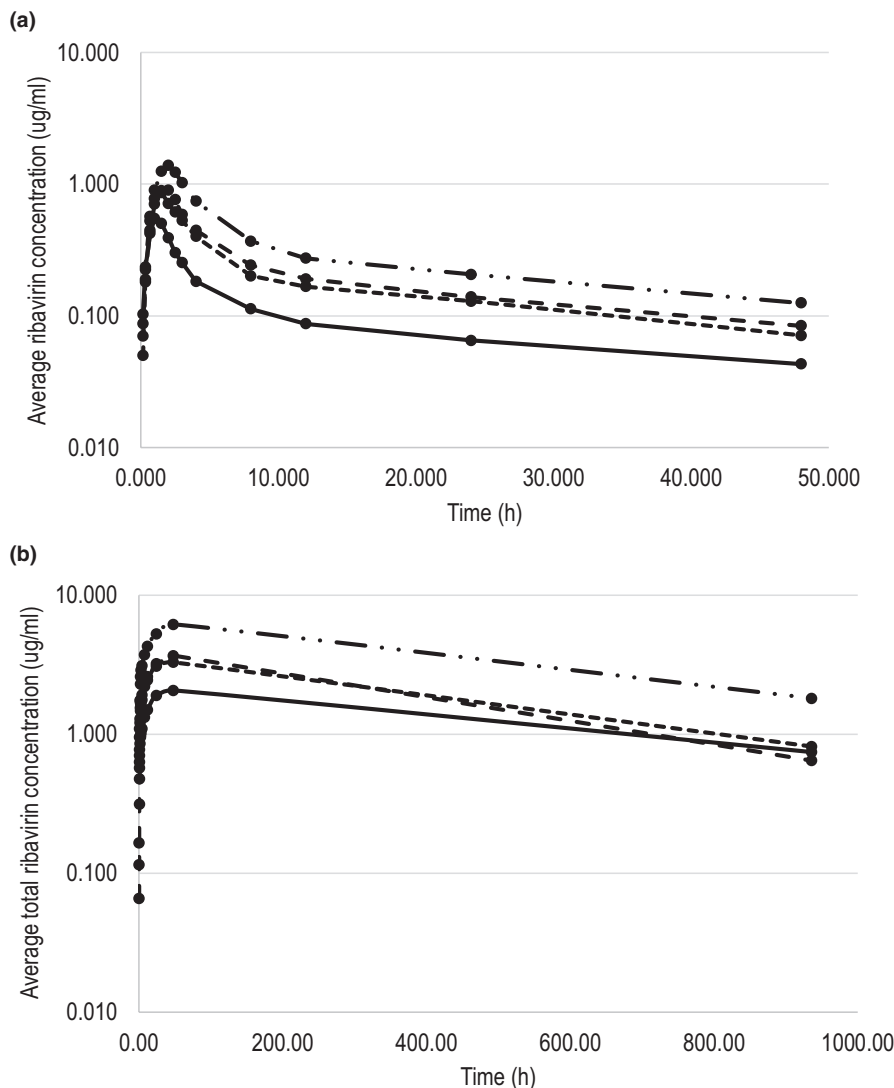


FIGURE 3 Semi-log plot of whole blood sample concentration–time profiles of (a) ribavirin and (b) total ribavirin after ascending doses of ribavirin aerosol up to 960 h post-dose in the active groups. Cohort 1: 50 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min). Cohort 2: 50 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min). Cohort 3: 100 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 10 ml total volume) aerosolized; administered until solution depleted (approximately 20 min). Cohort 4: 100 mg/ml ribavirin (active, $N = 6$) or placebo ($N = 2$; 20 ml total volume) aerosolized; administered until solution depleted (approximately 40 min). N , number of subjects.

TABLE 3 Pharmacokinetic parameters for ribavirin in plasma across active cohorts

Parameter	Cohort 1 ($N = 6$)	Cohort 2 ($N = 6$)	Cohort 3 ($N = 5$)	Cohort 4 ($N = 6$)
Plasma samples				
C_{max} ($\mu\text{g/ml}$)	0.63 ± 0.28 (44.9)	1.07 ± 0.35 (33.4)	0.95 ± 0.17 (17.8)	1.64 ± 0.58 (35.3)
AUC_{last} ($\mu\text{g/ml}$)*(h)	4.90 ± 2.49 (50.8)	10.55 ± 3.75 (35.5)	9.27 ± 2.11 (22.8)	15.55 ± 5.58 (35.8)
T_{max} (h)	1.000 (1.0–1.5)	1.750 (1.0–2.5)	1.500 (1.4–1.5)	2.009 (1.5–2.5)
Whole blood samples				
C_{max} ($\mu\text{g/ml}$)	0.58 ± 0.26 (44.8)	0.95 ± 0.34 (35.9)	0.87 ± 0.1 (15.70)	1.46 ± 0.48 (33.4)
AUC_{last} ($\mu\text{g/ml}$)*(h)	4.35 ± 2.33 (53.6)	9.38 ± 3.33 (35.4)	8.40 ± 1.94 (23.1)	13.96 ± 4.95 (35.4)
T_{max} (h)	1.00 (1.0–1.5)	1.75 (1.0–2.5)	1.50 (1.1–1.5)	2.00 (1.5–2.5)

Note: All data are presented as mean \pm standard deviation (coefficient of variation, %), except T_{max} , which is presented as median (minimum–maximum). Calculations for the placebo pharmacokinetic parameters could not be made since the ribavirin was below the level of quantitation (BLQ). Cohort 1: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 2: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 3: 100 mg/ml ribavirin (active) or placebo aerosolized. Cohort 4: 100 mg/ml ribavirin (active) or placebo aerosolized.

Abbreviations: AUC_{last} , area under the curve to the last measurable concentration; C_{max} , maximum observed concentration; N , number of subjects in the specified cohort/treatment group; T_{max} , time to reach C_{max} .

environmental contamination related to secondary exposure of aerosolized ribavirin²⁶ have been reported with aerosol use. We saw no clinically meaningful differences

between ribavirin aerosol treatment and placebo groups in the frequency or type of TEAEs in the current study. No AEs were reported in the subjects receiving placebo in

TABLE 4 Pharmacokinetic parameters for total ribavirin in plasma across active cohorts

Parameter	Total ribavirin in plasma			
	Cohort 1 (N = 3)	Cohort 2 (N = 6)	Cohort 3 (N = 5)	Cohort 4 (N = 6)
C_{max} (µg/ml)	0.68 ± 0.14 (20.98)	0.83 ± 0.28 (33.58)	0.74 ± 0.13 (18.03)	1.32 ± 0.46 (35.10)
AUC_{last} (µg/ml)*(h)	1.25 ± 0.43 (34.52)	2.54 ± 1.55 (60.88)	2.15 ± 0.96 (44.65)	5.96 ± 4.97 (83.37)
T_{max} (h)	1.00 (1.00–1.00)	1.50 (1.00–2.50)	1.50 (0.98–2.00)	2.01 (1.52–2.50)
Parameter	Total ribavirin in whole blood			
	Cohort 1 (N = 6)	Cohort 2 (N = 6)	Cohort 3 (N = 5)	Cohort 4 (N = 6)
C_{max} (µg/ml)	2.07 ± 0.79 (38.14)	3.68 ± 1.22 (33.16)	3.30 ± 0.57 (17.27)	6.19 ± 2.59 (41.87)
AUC_{last} (µg/ml)*(h)	937.29 ± 730.38 (77.93)	2082.73 ± 665.32 (31.94)	1948.17 ± 311.80 (16.01)	3786.87 ± 1585.74 (41.88)
T_{max} (h)	47.73 (46.18–49.02)	48.03 (47.33–49.42)	48.33 (24.02–48.92)	47.48 (46.35–48.28)

Note: All data are presented as mean ± standard deviation (coefficient of variation, %), except T_{max} , which is presented as median (minimum–maximum). Calculations for the placebo pharmacokinetic parameters could not be made since the ribavirin was below the level of quantitation (BLQ). Cohort 1: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 2: 50 mg/ml ribavirin (active) or placebo aerosolized. Cohort 3: 100 mg/ml ribavirin (active) or placebo aerosolized. Cohort 4: 100 mg/ml ribavirin (active) or placebo aerosolized.

Abbreviations: AUC_{last} , area under the curve to the last measurable concentration; C_{max} , maximum observed concentration; N, number of subjects in the specified cohort/treatment group; T_{max} , time to reach C_{max} .

cohorts 2 (20 ml of 50 mg/ml), 3 (10 ml of 100 mg/ml), and 4 (20 ml of 100 mg/ml). Although cohort 1 (active (10 ml of 50 mg/ml) and placebo groups reported three AEs (one in the active and two in the placebo group) and cohort 4 (active group, 20 ml of 100 mg/ml) reported four AEs, none were classified as serious or severe and none resulted in study or treatment discontinuation. The types of AEs reported were the same as those captured in the ribavirin product monograph, such as headache, nausea, vomiting, increased aspartate aminotransferase, elevated systolic blood pressure, and ventricular extra systole and they were all moderate or mild in severity.¹⁶ No DLTs were observed, and all dose escalations occurred as planned.

These results show that the ascending doses of ribavirin were all well-tolerated. This was anticipated as we used healthy subjects with a short treatment period of 20 or 40 min, once daily. This treatment time is much shorter than the currently approved pediatric use of ribavirin aerosol for RSV in which the patient receives as a continuous exposure to the ribavirin aerosol for 12–18 h per day for 3–7 days.¹⁵ The approved RSV dose of 20 mg/ml over 12 h leads to a calculated dose of 10.9 mg/kg.¹⁶ However, a single dose of ribavirin 100 mg/ml over 30 min results in a calculated dose of 5.1 mg/kg. This means shorter treatment periods, one-third to one-sixth the amount of ribavirin being administered, reduced environmental contamination and secondary exposure from the aerosol compared to the US Food and Drug Administration (FDA)-approved dose, and more time for other patient care needs.¹⁶ Even though our results are from healthy volunteers, a recent case series of five COVID-19 patients in Italy showed that ribavirin aerosol administered at 100 mg/ml for 30 min twice daily for 6 days resulted in

patients fully recovering with no reported AEs associated with ribavirin treatment.¹⁵

The PK results are promising, specifically in the context of coronavirus and the intensive care setting where patients' ability to swallow is compromised and delivery of a drug to the site of infection (respiratory tract) provides advantages over oral and intravenous formulations.²⁷ The C_{max} for ribavirin was achieved within 2 h across all doses which supports a relatively rapid exposure of ribavirin in the lung. The results appear linear and well-tolerated across the four single-dose regimens, demonstrating systemic exposure with minimal systemic effects. This indicates a favorable dose-exposure-effect relationship for ribavirin aerosol that could lend itself well to the coronavirus clinical setting. It is important to discuss subjects with BLQ concentrations. One subject from the active group (cohort 3) showed BLQ concentrations for ribavirin and total ribavirin in plasma and whole blood, which was unexpected and led to further investigation. Examination of records for investigational product (IP) dispensing, IP administration, blood and plasma processing, deviations log, and bioanalytical quality controls did not reveal any issues that could explain this observation. Closer examination of the bioanalytical data for this specific subject showed that albeit BLQ values were reported, the presence of the analytes could be detected for ribavirin and total ribavirin while being devoid of signal in the pre-dose sample and blank samples. This supports the subject having received the product and that a lower LLOQ would have allowed characterization of the profile. Pharmacokinetic outliers are uncommon but have been reported in the literature. In the specific case of ribavirin, pharmacokinetic of ribavirin shows high intra- and inter-individual variability. Such

variations have been attributed to genetic polymorphism present in genes coding transporters affecting ADME (Absorption, Distribution, Metabolism, and Elimination). It might be hypothesized that genetic polymorphism may have contributed to the low pulmonary absorption.

Cohort 1, active group, had three subjects with fewer than four consecutive concentrations for total ribavirin in plasma. This reflects a 10-fold difference in the LLOQ for total ribavirin compared to total ribavirin leading to an incomplete pharmacokinetic profile for total ribavirin in three subjects of cohort 1 whereas the profile could be characterized for all other analytes/matrices.

This study has some limitations. It was conducted in healthy volunteers, using a single dose of ribavirin, which limits extrapolation to clinical practice and patients with disease undergoing courses of treatment. Inclusion criteria were strict thus the impact of concurrent medications and/or diseases seen in real world clinical practice may affect safety and PK parameters. Regarding the safety assessments, hematologic changes in RBCs can take place up to 40 days after dosing therefore impact on blood cell counts may not have been observed during the window period in between day 3 to day 40 assessments due to the absence of interim safety assessments. For the PK assessments, it is acknowledged that different bioanalytical methods for total ribavirin and ribavirin in plasma have been developed and validated independently with different internal standards and LOQs. This results in differences observed in AUC between total ribavirin and ribavirin in plasma that reflects the difference in bioanalytical method. The LOQ is approximately 10-fold higher for total ribavirin versus ribavirin in plasma leading to several timepoints being BLQ for total ribavirin. The later has a direct impact on the total ribavirin calculated value, resulting in lower AUC values for total ribavirin. In contrast, C_{max} is not sensitive to the difference in LLOQs therefore the observed C_{max} values are comparable for total ribavirin versus ribavirin for all cohorts. The analytical methods have been developed and validated in compliance with the acceptance criteria of the FDA and other regulatory guidelines. Lastly, the study was conducted in one Canadian health care center thus limiting the applicability to other health care systems and countries. However, this was a phase Ia safety and PK study to evaluate a clinically relevant administration protocol of ribavirin aerosol. The results are promising and show that further study is warranted in the clinical setting.

CONCLUSIONS

In conclusion, ribavirin inhalation treatment was found to be safe and well-tolerated in healthy adult subjects, without DLTs across a dose range of 50 to 100 mg/ml, and

with a comparable safety profile to placebo. The PK results appeared linear and well-tolerated across the four single-dose regimens, demonstrating systemic exposure with minimal systemic effects. These results support the continued clinical development of ribavirin aerosol as a treatment option in patients with coronaviruses.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. P.C., A.B., J.L.V., R.J.I., and M.B. designed the research, performed the research, and analyzed the data. C.P. and J.P. contributed new reagents/analytical tools.

ACKNOWLEDGMENTS

Medical writing assistance for this manuscript was provided by KTP (Knowledge Translation Partners), Montreal, Canada, funded by Bausch Health Canada. Statistical analyses were conducted by Clianza Research.

CONFLICT OF INTEREST

A.B.: employee and ownership of stocks and shares in Bausch Health. J.L.V.: employee and ownership of shares in Bausch Health. M.B.: employee and ownership of shares in Bausch Health. R.J.I.: employee and ownership of shares in Bausch Health. All other authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Couroux P, Brkovic A, Vittitow JL, et al. A randomized, placebo-controlled study to evaluate safety and pharmacokinetics of inhaled ribavirin. *Clin Transl Sci.* 2022;15:2159-2171. doi: [10.1111/cts.13350](https://doi.org/10.1111/cts.13350)