ARTICLE

Safety, Tolerability, and Pharmacokinetics of Remdesivir, An Antiviral for Treatment of COVID-19, in Healthy Subjects

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Remdesivir (RDV), a single diastereomeric monophosphoramidate prodrug that inhibits viral RNA polymerases, has potent *in vitro* antiviral activity against severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). RDV received the US Food and Drug Administration (FDA)'s emergency use authorization in the United States and approval in Japan for treatment of patients with severe coronavirus disease 2019 (COVID-19). This report describes two phase I studies that evaluated the safety and pharmacokinetics (PKs) of single escalating and multiple i.v. doses of RDV (solution or lyophilized formulation) in healthy subjects. Lyophilized formulation was evaluated for potential future use in clinical trials due to its storage stability in resource-limited settings. All adverse events were grade 1 or 2 in severity. Overall, RDV exhibited a linear profile following single-dose i.v. administration over 2 hours of RDV solution formulation across the dose range of 3–225 mg. Both lyophilized and solution formulations provided comparable PK parameters. High intracellular concentrations of the active triphosphate (~ 220-fold to 370-fold higher than the *in vitro* half-maximal effective concentration against SARS-CoV-2 clinical isolate) were achieved following infusion of 75 mg or 150 mg lyophilized formulation over 30 minutes or 2 hours. Following multiple-doses of RDV 150 mg once daily for 7 or 14 days, RDV exhibited a PK profile similar to single-dose administration. Metabolite GS-441524 accumulated ~ 1.9-fold after daily dosing. Overall, RDV exhibited favorable safety and PK profiles that supported once-daily dosing.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Currently, there are no US Food and Drug Administration (FDA) approved drugs for treatment or prevention of coronavirus disease 2019 (COVID-19). Supportive care is the mainstay therapy for patients with COVID-19. Several antiviral therapies are being investigated for treatment potential. RDV is under regulatory review for the treatment of COVID-19.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This was a phase I, first-in-human (FIH) study that assessed the safety, tolerability, and pharmacokinetics (PKs) of remdesivir (RDV) in healthy volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study demonstrated that PK of RDV, as both solution and lyophilized formulation, supports once-daily dosing and was well-tolerated in healthy volunteers.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ RDV FIH study data, in addition to nonclinical data, supported further clinical development of RDV in treatment of COVID-19.

Coronaviruses (CoVs) are positive-sense, single-stranded, enveloped RNA viruses with high mutation and recombination rates, which allow them to cross species barriers and adapt to new hosts.[1–3] The genetically diverse CoV family, comprised of four groups: alpha-CoV, beta-CoV, gamma-CoV, and delta-CoV, infects a variety of avian and mammalian species (such as camels, cats, and bats).[1,4–6] Human CoVs generally cause mild to moderate upper-respiratory tract illnesses. However, recent years have seen the emergence of more virulent strains, such as severe acute respiratory syndrome coronavirus (SARS-CoV) and

Middle Eastern respiratory syndrome coronavirus, that can cause life-threatening illness.[4,6,7]

In December 2019, SARS-CoV-2 was identified as the cause of an outbreak of respiratory illness in Wuhan, China. SARS-CoV-2 shared 79% sequence identity with SARS-CoV and both viruses utilized the angiotensin converting enzyme II receptor for cell entry.[8,9] This outbreak was declared a Public Health Emergency of International Concern by the World Health Organization (WHO) on January 30, 2020.[10] The clinical disease termed coronavirus disease 2019 (COVID-19) is highly transmissible. The incubation

period of COVID-19 ranges from 1–14 days. Common clinical symptoms include fever, fatigue, dry cough, shortness of breath, and myalgia.[11,12] In severe cases, COVID-19 can cause pneumonia, acute respiratory distress syndrome, organ failure, septic shock, and death.[13]

Worldwide there are over 7.9 million infections with over 430,000 deaths related to COVID-19.[14] There are currently no approved effective therapeutic agents available for the treatment of COVID-19. Remdesivir (RDV; GS-5734) is a diastereomeric monophosphoramidate prodrug, which undergoes metabolic activation to form intracellularly the active triphosphate, GS-443902. Briefly, RDV is extensively metabolized by hydrolase activity and forms an intermediate metabolite, GS-704277, which then is subject to cleavage of the phosphoramidate bond resulting in the formation of the nucleoside analog monophosphate, which is further phosphorylated to the pharmacologically active nucleoside triphosphate, GS-443902, that selectively inhibits viral RNA polymerases but not host RNA or DNA polymerases (**Figure S1**).[15–17] Dephosphorylation of the nucleoside analog monophosphate results in the formation of the nucleoside analog, GS-441524, that is not efficiently re-phosphorylated. RDV and its metabolites (GS-441524 and GS-704277) are detectable in plasma. Because all target cells relevant for SARS-CoV-2 infection are not currently known, peripheral blood mononuclear cells (PBMCs) are used as the surrogate to assess the intracellular activation of RDV to GS-443902.

In vitro studies in different cell types demonstrated that RDV and GS-441524 are active against SARS-CoV-2, with potency directly related to the intracellular concentrations of GS-443902. *In vitro*, RDV exhibited antiviral activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial cells with an half-maximal effective concentration (EC_{50}) of 9.9 nM after 48 hours of treatment.[18] In Vero cells, the EC_{50} values of RDV were 137 nM at 24 hours and 750 nM at 48 hours post-treatment.[19] In SARS-CoV-2-infected rhesus monkeys who received i.v. bolus injections of 10 mg/kg on the first day (initiated 12 hours post-inoculation), followed by 5 mg/kg daily thereafter, RDV resulted in a significant reduction in clinical scores, signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals.[4,19–21]

On May 1, 2020, based on available data from two global clinical trials, the US Food and Drug Administration (FDA) granted emergency use authorization to allow RDV to treat adults and children hospitalized with severe COVID-19.[19,22,23] Based on these clinical data, RDV has been approved for the treatment of adults and pediatric patients in Japan.[24] This paper describes the safety and pharmacokinetics (PKs) of the solution and lyophilized formulations of i.v. RDV administered to healthy participants in the two first-in-human (FIH) phase I studies.

METHODS
Study population
Protocols and informed consents for both studies were approved by the study center's institutional review board, and subjects provided written consent before study participation. The single-dose and multiple-dose studies were randomized, blinded, placebo-controlled, phase I studies to evaluate the PK, safety, and tolerability of single-ascending (study 1) or multiple (study 2) i.v. doses of RDV compared with placebo in healthy subjects conducted at SeaView Research, Miami, FL. Eligible subjects were healthy male and nonpregnant, nonlactating female subjects of non-childbearing potential, 18–55 years of age with a body mass index between 18 and 30 kg/m^2. Subjects did not participate in more than one cohort of the study.

Major inclusion criteria included healthy subjects based on medical history/physical examinations/laboratory evaluations, normal 12-lead electrocardiogram (ECG), creatinine clearance > 90 mL/min, no evidence of HIV, hepatitis B virus or hepatitis C virus infection, and use of at least 2 forms of contraception, including an effective barrier method. Exclusion criteria included plasma and blood donation within 7 and 56 days of study entry, respectively, active medical illness, use of prescription drugs within 28 days of study drug dosing (except vitamins, acetaminophen, ibuprofen, and/or hormonal contraceptive).

Study design
Single-dose study. In study 1, 9 dose cohorts were evaluated. In cohorts 1–6, eligible subjects were randomized 4:1 within each cohort to receive RDV solution formulation at doses ranging from 3 mg to 225 mg (*n* = 8) or placebo (*n* = 2) on day 1, administered as a 2-hour infusion. In cohorts 7–9, eligible subjects were randomized 5:1 (*n* = 10 active: *n* = 2 placebo) within each cohort to receive RDV lyophilized formulation at single doses of either 75 mg (cohort 7 and cohort 9) or 150 mg (cohort 8) or placebo on day 1. Subjects in cohorts 7 and 8 received a single i.v. dose of study medication administered over a 2-hour period, whereas subjects in cohort 9 received a single i.v. dose of RDV administered over a 30-minute period. Dose escalation in cohorts 1–6 occurred in sequential order after reviewing the safety data from the previous cohort, and only in the absence of dose-limiting toxicity and/or meeting any prespecified stopping criteria. Cohorts 7 and 8 were conducted in parallel and informed the conduct of cohort 9. Subjects were discharged on day 7 and returned 6 days (+/- 1 day) later for an in-clinic follow-up visit.

Multiple-dose study. In study 2, two cohorts were enrolled and sentinel dosing was utilized within each cohort. Subjects were randomized 2:1 (8 active: 4 placebo) within each cohort to receive RDV 150 mg or matching placebo, respectively. Each cohort was comprised of 2 groups of 6 subjects each (4 who received RDV and 2 who received placebo). Each subject received study drug administered i.v. over a 1-hour period once daily for 7 days in cohort 1 and 14 days in cohort 2. Subjects were discharged on day 9 for cohort 1 and day 16 for cohort 2. All subjects returned 7 days (+/- 2 day) after discharge for an in-clinic follow-up visit.

Dose selection. The starting dose of 3 mg in study 1 (single dose) was selected in accordance with the FDA guidance document for estimating a safe starting dose of a new chemical entity in human test subjects[25] and was expected to provide a 15 and 65-fold safety margin relative to the no-observed-adverse-effect levels from the 14-day toxicology

studies in rat and cynomolgus monkey, respectively (data on file). At the monkey no-observed-adverse-effect level, the species that more closely mimics the behavior of RDV in humans in terms of plasma stability, anticipated margins of exposures (area under the plasma concentrations-time curve (AUC)) for RDV and GS-441524 were at least 70-fold and 75-fold at the 3 mg dose. Single doses up to 225 mg evaluated in this study were guided by emerging safety and PK data from lower dose cohorts. The evaluated dose range allowed for large dose separation for assessment of safety and PK. The dose of 150 mg in study 2 (multiple dose) was supported by the 14-day toxicology data (data on file) and the favorable safety profile in healthy participants who received single doses up to 225 mg in study 1. The 150 mg dose was projected to achieve systemic exposures similar to exposures expected in the infected rhesus monkeys treated with therapeutic doses of RDV 10 mg/kg i.v.[20]

Pharmacokinetic evaluation
Subjects in cohorts 1–6 were administered RDV solution formulation over a 2-hour period, subjects in cohorts 7 and 8 were administered RDV lyophilized formulation over a 2-hour period, and subjects in cohort 9 were administered RDV lyophilized formulation over a 30-minute period. Serial blood samples were collected prior to dosing and postdose PK sampling was performed over 144 hours. Urine samples were collected prior to dosing and relative to the start of infusion on day 1 over 48 hours. Blood samples for PBMC isolation was collected at predose and over 144 hours relative to the start of infusion.

For the multiple-dose study, intensive PK sampling occurred on days 1 and 7 for cohort 1, and days 1, 7, and 14 for cohort 2 prior to dosing, and postdose PK sampling was performed over 24 hours. On the last day of dosing for each cohort (day 7 of cohort 1 or day 14 of cohort 2), 36-hour and 48-hour samples were also collected. Additionally, blood sample(s) were collected prior to dosing on day 4 of cohorts 1 and 2 and day 11 of cohort 2.

Bioanalytical procedures
Plasma concentrations of RDV, GS-704277, and GS-441524 were determined using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method with multiple reaction monitoring and electrospray ionization in the positive mode (QPS; LLC, Newark, DE). Quantification was performed using multiple reaction monitoring of the transitions m/z $603.3 \rightarrow 402.2$ and m/z $606.3 \rightarrow 402.2$ for RDV and an isotopically labeled internal standard (GS-829143), m/z $441.1 \rightarrow 150.1$ and m/z $444.1 \rightarrow 150.1$ for GS-704277, and an isotopically labeled internal standard (GS-829466), m/z $292.2 \rightarrow 202.2$ and m/z $295.2 \rightarrow 205.2$ for GS-441524, and an isotopically labeled internal standard (GS-828840), respectively. The bioanalytical method was validated over the calibrated ranges of 4–4000 ng/mL for RDV, 2–2000 ng/mL for GS-704277, and 2–2000 ng/mL for GS-441524, respectively. Interassay precision, based on coefficient of variation (%CV) for RDV, GS-704277, and GS-441524, ranged from 2.1–5.3%, and accuracy, based on interassay percent relative error (%RE) for RDV, GS-704277, and GS-441524,

ranged from –9.8% to 9.5%. All samples were analyzed in the timeframe supported by frozen stability storage data.

Urine concentrations of RDV and GS-441524 were determined using a similar validated LC-MS/MS bioanalytical method as plasma method except that the analysis of GS-704277 in urine was not conducted because a validated bioanalytical method was not available at the time of sample analysis. The bioanalytical method was validated over the calibrated ranges of 10–5,000 ng/mL for both RDV and GS-441524. Interassay precision for RDV and GS-441524 ranged from 1.7%CV to 4.4%CV, and accuracy for RDV and GS-441524, ranged from –6.8%RE to 1.0%RE. All samples were analyzed in the timeframe supported by frozen stability storage data.

PBMC concentrations of GS-441524 (total concentrations) in cohorts 1–6 were determined using a validated LC-MS/MS method at QPS. Total concentrations of GS-441524 is defined as the sum of the endogenous concentration of GS-441524 plus concentrations of GS-441524 resulting from enzymatic dephosphorylation of its phosphorylated metabolites in human PBMCs. This method involved cell lysis extraction of GS-441524 and its phosphorylated metabolites followed by enzymatic dephosphorylation of its phosphorylated metabolites to GS-441524. The enzymatic dephosphorylation procedure was established using GS-443902 (GS-441524-tri-phosphate) as the representative substrate because both monophosphate and diphosphate were unavailable. Its mean dephosphorylation efficiency, calculated by dividing the mean concentration of GS-441524 by the nominal concentration from the 3 precision and accuracy runs, was determined to be 70.3%, which was used as acceptance criteria for enzymatic dephosphorylation procedure. Quantification was performed using multiple reaction monitoring of the transitions m/z $292.2 \rightarrow 202.2$ for GS-441524 and m/z $306.4 \rightarrow 216.1$ for an internal standard (GS-441285). The bioanalytical method was validated over the calibrated range of 4–2,000 nmol/L. Interassay precision and accuracy ranged from –2.4%CV to 7.2%CV, and from –0.6%RE to 5.8%RE, respectively. All samples were analyzed in the timeframe supported by frozen stability storage data.

PBMC concentrations of GS-443902 (the active triphosphate) in cohorts 7–9 were determined using a validated LC-MS/MS method. This method involved cell lysis extraction of GS-443902 and its isotopically labeled internal standard (GS-829492) from human PBMCs. Quantification was performed using multiple reaction monitoring of the transitions m/z $532.2 \rightarrow 202.2$ for GS-443902 and m/z $535.0 \rightarrow 205.0$ for an isotopically labeled internal standard (GS-829492). The bioanalytical method was validated over the calibrated range of 5–2,500 ng/mL. Interassay precision and accuracy ranged from 1.8%CV to 4.5%CV, and from –10.0%RE to –0.9%RE, respectively. All samples were analyzed in the timeframe supported by frozen stability storage data.

Safety analyses
During and following dosing, safety and tolerability were assessed through the reporting of treatment-emergent

adverse events (AEs), clinical laboratory tests (hematology profile, chemistry profile, and urinalysis) according to the Division of Acquired Immunodeficiency Syndrome (DAIDS) AEs grading table, version 1.0, physical examinations, vital signs, serum pregnancy tests (female subjects) review of concomitant medications, and ECGs at various time points during the study.

PK analyses

PK parameters were estimated using noncompartmental methods using a linear-up/log-down method for AUC estimation (WinNonlin 6.3; Pharsight, St. Louis, MO). PK parameters assessed in both studies included AUC from time zero to the last quantifiable concentration (AUC_{last}), the AUC vs. time curve extrapolated to infinity (AUC_{inf}), the AUC over a dosing interval (at steady state; AUC_{tau}), maximum observed plasma concentration (C_{max}), and time of occurrence of C_{max} (T_{max}), terminal elimination half-life ($t_{1/2}$), clearance (CL), renal clearance (CL_r), and volume of distribution.

Statistical analyses

For both studies, the PK analysis set included all subjects who received at least one dose of study drug and had at least one nonmissing postdose concentration. The safety population included all subjects who received at least one dose of RDV or placebo.

No formal power or sample size calculations were used to determine cohort size in these phase I studies. Subject demographics and baseline characteristics, plasma, PBMC, urine PK parameters, and safety data were summarized by descriptive statistics for continuous data and by number and percentage of subjects for categorical data.

Dose proportionality was obtained by comparing the values of the RDV, GS-774277, and GS-441524 parameters across the evaluated doses. Dose proportionality was evaluated using both the power model and the analysis of variance fitted to the natural logarithmic transformation of PK parameters (AUC_{inf}, AUC_{last}, and C_{max}) for all analytes. Due to the exploratory nature of the study, no dose proportionality boundaries have been prespecified. In the multiple-dose study, accumulation of RDV and metabolites was assessed by comparing day 7 to day 1 PK for cohort 1, and day 14 to day 1 PK for cohort 2. A time to achieve to steady-state of RDV and metabolites was examined using the Helmert transformation testing procedure of trough plasma concentrations.[26]

RESULTS

Subject demographics and baseline characteristics

In the single-dose study, 96 subjects were randomized and all subjects completed the study. Of those, 78 subjects received RDV and 18 received placebo. These subjects had a mean age of 44 years (range 24–55 years). The majority of subjects (active + placebo) were men (58.3%), white (88.5%), and Hispanic or Latino (96.9%). In the multiple-dose study, 24 subjects were randomized of whom 16 subjects received RDV and 8 subjects received placebo. Twenty-two subjects completed the study. Two subjects, one from cohort 1 who received RDV and one from cohort 2 who received placebo,

prematurely discontinued study drug and the study due to AEs and withdrawal of consent, respectively. Subjects had a mean age of 44 years (range 19–55 years). The majority of subjects were male (58.3%). Most subjects (83.3%) were white, and all were Hispanic or Latino. For both studies, baseline characteristics were generally balanced across cohorts and are presented in **Table 1**.

Safety

Study drug was generally well-tolerated in both studies. There were no serious AEs, grades 3 or 4 AEs, or deaths reported in either study.

Single-dose escalation study

All AEs reported during this study were grade 1 or 2 in severity. There were no AEs leading to study drug or study discontinuation. A total of 17 of 78 subjects (21.8%) who received RDV and 2 of 18 subjects (11.1%) who received placebo had at least 1 AE. Constipation was the only AE reported for > 1 subject occurring in 3 of 78 subjects (3.8%) who received RDV. Two subjects receiving RDV solution formulation experienced AEs considered to be study drug related (dizziness, cohort 2; pruritus, cohort 6). Five subjects (4 received RDV solution formulation; 1 received RDV lyophilized formulation) had an AE considered related to study procedures. These were infusion site extravasation and medical device site dermatitis (each 1 subject in the 3-mg solution formulation group), ecchymosis (1 subject in the 10-mg solution formulation group), presyncope (1 subject in the 75-mg solution formulation group), and medical device site irritation (1 subject in the 75-mg lyophilized formulation/30 minute infusion group). All AEs related to study drug and procedures resolved.

The majority of laboratory abnormalities were grade 1 (35/78 (44.9%) received RDV; 5/18 (27.8%) received placebo) or grade 2 in severity (12/78 (15.4%) received RDV; 5/18 (27.8%) received placebo). Grade 2 laboratory abnormalities observed in > 1 subject overall were elevated total cholesterol (6/78 (7.7%) received RDV; 2/18 (11.1%) received placebo), and elevated low-density lipoprotein (LDL) cholesterol (6/78 (7.7%) received RDV; 3/18 (16.7%) received placebo). All subjects with grade 2 elevated total and LDL cholesterol, with the exception of 1 subject in the placebo group, had a grade 1 elevation in total and/or LDL cholesterol predose (day −1). One subject who received 225 mg RDV solution formulation had a grade 3 elevated lipase (225 U/L) on day 2 (baseline 29 U/L); this was a single occurrence, and values returned to within the normal range by day 5. This subject also had a grade 1 amylase value (133 U/L) on day 2 without evidence of clinical pancreatitis. No subject had a grade 4 laboratory abnormality during the study. Overall, no consistent patterns of laboratory abnormalities or changes from baseline in laboratory parameters were noted during the study. No patterns of clinically relevant changes in vital signs or shifts in 12-lead ECGs were observed during the study.

Multiple-dose study

All AEs were grade 1 in severity. A total of 9 of 16 subjects (56.3%) who received RDV and 4 of 8 subjects (50.0%)

Table 1 Subject demographics and baseline characteristics

Characteristics single-dose escalating study	Overall active (*N* = 78)	Pooled placebo (*N* = 18)	Overall (*N* = 96)
Mean age (range)	44 (24–55)	48 (35–55)	45 (24–55)
Sex			
Male	47 (60.3%)	9 (50.0%)	56 (58.3%)
Female	31 (39.7%)	9 (50.0%)	40 (41.7%)
Race			
White	69 (88.5%)	16 (88.8%)	85 (88.5%)
Black or African American	9 (11.5%)	2 (11.1%)	11 (11.5%)
Ethnicity			
Hispanic or Latino	76 (97.4%)	17 (94.4%)	93 (96.9%)
Not Hispanic or Latino	2 (2.6%)	1 (5.6%)	3 (3.1%)
Mean BMI, kg/m^2 (range)	26.8 (21.6–30.2)	27.2 (23.1–30.2)	26.9 (21.6–30.2)
Mean eGFR$_{CG}$, mL/min (range)	115.2 (90.0–172.2)	120.6 (96.6–162.8)	116.2 (90.0–172.2)
Characteristics multiple-dose study	**Cohort 1 (*N* = 8)**	**Cohort 2 (*N* = 8)**	**Pooled placebo (*N* = 8)**
Mean age (range)	44 (19–55)	42 (27–54)	42 (28–53)
Sex			
Male	4 (50.0%)	5 (62.5%)	5 (62.5%)
Female	4 (50.0%)	3 (37.5%)	3 (37.5%)
Race			
White	7 (87.5%)	8 (100%)	5 (62.5%)
Black or African American	1 (12.5%)	0	3 (37.5%)
Ethnicity			
Hispanic or Latino	8 (100.0%)	8 (100.0%)	8 (100.0%)
Not Hispanic or Latino	–	–	–
Mean BMI, kg/m^2 (range)	26.1 (20.6–29.3)	28.1 (26.6–31.8)	26.7 (24.0–28.8)
Mean eGFR$_{CG}$, mL/min (range)	111.1 (94.4–141.1)	121.2 (98.8–165.7)	120.8 (103.6–188.7)

BMI, Body mass index; eGFR$_{CG}$, estimated glomerular filtration rate calculated using the Cockcroft-Gault method.

who received placebo reported at least 1 AE. The AEs reported for > 1 subject overall included constipation, dyspepsia, and pain in extremity (each in 3 subjects who received RDV) and headache, dermatitis contact, and pruritus (each in 1 subject who received RDV and 1 subject who received placebo). Nine subjects (6 received RDV and 3 received placebo), had ≥ 1 AE that was considered related to study procedure. These AEs included pain in the extremities (3 subjects who received RDV); contact dermatitis and pruritus (each in 1 subject who received RDV and 1 who received placebo); infusion site extravasation, infusion site pain, infusion site hemorrhage, and ecchymosis (each in 1 subject who received RDV); and dermatitis and iron deficiency anemia (each in 1 subject who received placebo).

Four subjects (3 received RDV; 1 received placebo) had AEs that were considered by the investigator to be study drug related. In cohort 1, one subject who received RDV discontinued the study drug due to nausea and also experienced treatment-related headache, vomiting, and tremor. Additional treatment-related AEs of decreased appetite and constipation occurred after study drug discontinuation. This subject also experienced the concomitant laboratory abnormalities of grade 2 elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All AEs and graded abnormalities resolved. Two subject in cohort 2 who

received RDV had dyspepsia, both of which resolved. One of these subjects also had elevated ALT and AST levels, which returned to within normal range. One subject (cohort 2) who received placebo had diarrhea, which also resolved during the conduct of the study.

The majority of laboratory abnormalities were grade 1 or 2 in severity (14/16 (87.5%) receiving RDV; 6/8 (75.0%) receiving placebo). No subjects had a grade 3 or 4 laboratory abnormality during the study. Eight subjects (2 in cohort 1; 6 in cohort 2) who received RDV had treatment-emergent graded ALT elevations (maximum toxicity grade 2 for 4 subjects; grade 1 for 4 subjects). Of these 8 subjects, 7 also had a graded AST elevation (1 subject in cohort 1 had a grade 2 elevation, and 6 subjects in cohort 2 had grade 1 AST elevation). One subject who received placebo in cohort 2 had a grade 1 ALT elevation. For all subjects with graded transaminase elevations, ALT and AST values returned to within the normal range during the study. Time to resolution ranged from 3 days (while still receiving study drug) to 47 days (43 days after completing study drug). None of the nine subjects with graded transaminase elevations had abnormalities in total bilirubin, alkaline phosphatase, or albumin during the study. Seven of the subjects with transaminase elevations also had grade 1 increased prothrombin time. All graded prothrombin time increases resolved to within the normal range during the

study for all subjects. No clinically significant change in International Normalized Ratio occurred in the seven subjects with graded prothrombin time increase. The other treatment-emergent grade 2 laboratory abnormality observed in > 1 subject was elevated total cholesterol, which was observed in 2 of 8 subjects in the pooled placebo group. No patterns of clinically relevant changes from predose in vital signs or shifts in 12-lead ECGs were observed during the study and no clinically significant changes in ECGs were observed.

Pharmacokinetics

Single-dose study. The single dose plasma concentration-vs-time profiles of RDV, its intermediate metabolite GS-704277, and the nucleoside metabolite GS-441524 are shown in **Figure 1**. The PK parameters for cohorts 1–6 are summarized in **Table 2** and for cohorts 7–9 are summarized in **Table 3**. Following a 2-hour i.v. infusion of the solution formulation (cohorts 1–6), RDV plasma concentrations declined rapidly and were accompanied by sequential appearance of GS-704277 (T_{max}, 1.97–2.25 hours; $t_{1/2}$, 0.87–1.8 hours), and the nucleoside metabolite GS-441524 (T_{max}, 3.5–5.0 hours; $t_{1/2}$, ~13–31 hours), the predominant plasma metabolite of RDV. The mean renal CL (CL_r) of RDV (48.6–78.1 mL/min) and GS-441524 (116–154 mL/min), and percentage of the RDV dose recovered in urine as unchanged drug (7.4% to 9.9%) over the 48-hour collection period was comparable across the 30-mg to 225-mg dose groups. Analysis of GS-704277 in urine was not conducted because a validated bioanalytical method was not available at the time of sample analysis.

Dose proportional increases in the exposures (AUC_{last}, AUC_{inf}, and C_{max}) of RDV and metabolites were observed across the evaluated dose range (3, 10, 30, 75, 150, and 225 mg) using both power model and analysis of variance analyses. Comparison of the solution (cohorts 4 and 5) and lyophilized formulations (cohorts 7 and 8) revealed comparable PK profiles of RDV and metabolites at the 75 mg and 150 mg doses following a 2-hour infusion. Like the solution formulation, dose proportional increases in plasma exposures of all analytes were noted for the lyophilized formulation.

The effect of duration of infusion on plasma PK of RDV and metabolites was evaluated following single-dose i.v. administration of RDV 75-mg lyophilized formulation over 30 minutes in cohort 9. The overall RDV exposures (AUC_{inf} and AUC_{last}) were in the range of those observed in the corresponding group that received the same 75-mg dose of the lyophilized formulation over 2 hours (cohort 7). As expected, the C_{max} of RDV increased upon shortening of infusion time from 2 hours to 30 minutes. The exposure parameters of GS-704277 and GS-441524 were similar in the lyophilized formulation 75-mg dose groups infused over 30 minutes and 2 hours.

Drug distribution into PBMCs following single dose i.v. RDV was assessed by measuring the total concentrations of GS-441524, which served as a surrogate for total intracellular phosphorylated species (cohorts 1–6), or the active triphosphate GS-443902 concentrations (cohorts 7–9). The total GS-441524 exposures in PBMCs increased in a dose-proportional manner across the evaluated dose range. The median $t_{1/2}$ of GS-441524 in PBMCs was comparable across the 3 to 225-mg dose range (32 to 48 hours; data on file). High intracellular concentrations of the active triphosphate, GS-443902, have been observed at 24 hours postdose following infusion of 75 mg or 150 mg lyophilized formulation over 30 minutes or 2 hours. These concentrations were ~ 220 to 370-fold above the *in vitro* EC_{50} against SARS-COV-2 (clinical isolate; $EC_{50} = 0.0099$ μM). The median $t_{1/2}$ of GS-443902 in PBMCs was comparable (36–49 hours) across all cohorts (**Table 4**).

Multiple-dose study. The day 7 and day 14 plasma PK parameters of RDV, its intermediate metabolite GS-704277, and the nucleoside metabolite GS-441524 following multiple 150-mg doses of RDV administered as 1-hour i.v. infusions were pooled together across both cohorts and are presented in **Table 5** and the concentration-vs-time profiles are presented in **Figure 2**. Following once-daily i.v. administration of 150-mg doses of RDV for 7 or 14 days, RDV had a median $t_{1/2}$ of ~ 1 hour, and accumulation did not occur given the short half-life. The nucleoside analog metabolite GS-441524 had a longer median $t_{1/2}$ of ~24.5 hours, with an accumulation ratio for geometric least squares mean of AUC after multiple daily dosing of ~ 1.9, reaching steady-state by day 4. The intermediate metabolite GS-704277 had a median $t_{1/2}$ of ~1.7 hours. Accumulation of this metabolite was observed after multiple dosing (accumulation ratio for geometric least squares mean of AUC of ~ 1.4–1.9), a finding which is inconsistent with its short half-life. Based on the principle of superpositioning, no accumulation of GS-704277 is expected.

DISCUSSION

The severe illness caused by COVID-19 underlines the urgent need to develop therapeutic and prophylactic agents as there are no approved vaccines or therapies.[27–29] Development of RDV for COVID-19 treatment was supported by its preclinical characteristics, including nonclinical safety and tolerability profiles and potent antiviral activity (*in vitro* and *in vivo* activity against SARS-CoV-2 and multiple genetically diverse CoVs).[4,17,20,30]

This paper reports results of FIH single-dose and multiple-dose studies conducted to evaluate safety and PK of solution and lyophilized formulations of RDV in healthy volunteers. The solution formulation provides a ready-to-use dosage form that does not require reconstitution prior to administration; thereby, simplifying the preparation at the administration site. The lyophilized formulation allows for longer-term storage compared with the refrigerated storage for the liquid formulation and is well-suited to address immediate needs in outbreak situations. Both formulations have been used in a clinical setting.

Overall, single-dose i.v. administration of RDV as a solution or lyophilized formulation for up to 2 hours at doses ranging from 3–225 mg and multiple-dose i.v. administration of RDV 150 mg once daily for 7 or 14 days was generally well-tolerated. All AEs reported during the single-dose study were grade 1 or 2 in severity. No subject had a graded ALT or
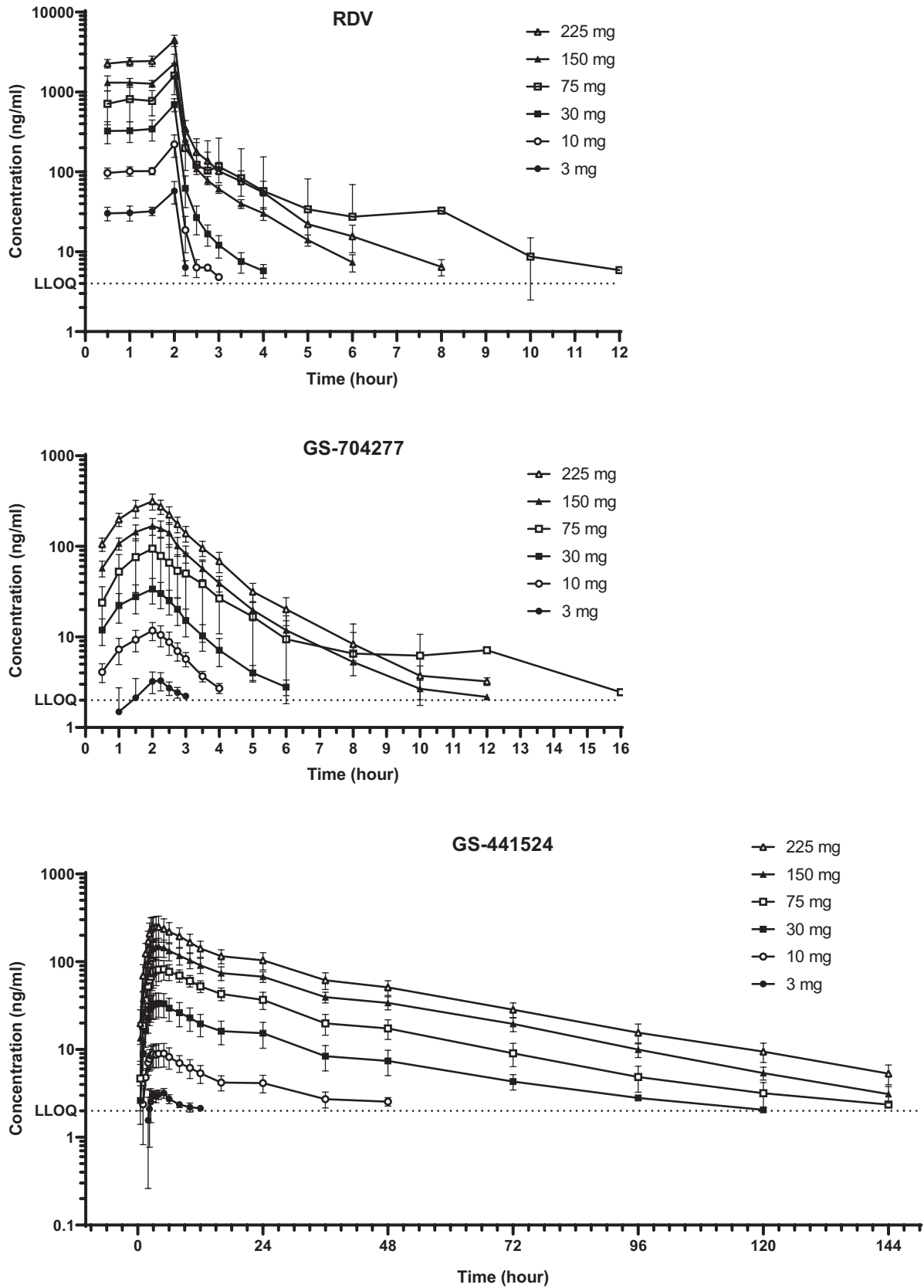
Figure 1 Plasma concentration-vs-time profiles following RDV single-dose administration; mean (±SD) values are plotted. LLOQ, lower limit of quantification; RDV, remdesivir.

Table 2 Pharmacokinetic parameters of RDV, GS-441524, and GS-704277 in cohorts 1–6 of the single-ascending-dose study

Parameter (RDV solution; 2-hour infusion)	Cohort 1 3 mg N = 8	Cohort 2 10 mg N = 8	Cohort 3 30 mg N = 8	Cohort 4 75 mg N = 8	Cohort 5 150 mg N = 8	Cohort 6 225 mg N = 8
RDV						
AUC_{inf}, h·ng/mL	–	230 (28.4)[a]	774 (22.9)	2000 (27.1)	2980 (19.0)	5270 (11.6)
AUC_{last}, h·ng/mL	67.1 (17.2)	230 (16.1)	768 (23.2)	1990 (27.3)	2970 (19.1)	5260 (11.7)
C_{max}, ng/mL	57.5 (31.1)	221 (31.2)	694 (18.6)	1630 (38.6)	2280 (30.1)	4420 (16.0)
T_{max}, hours	2.03 (2.01–2.04)	2.01 (2.00–2.03)	2.02 (2.00–2.03)	2.03 (2.03–2.05)	2.00 (1.98–2.04)	1.97 (1.95–1.98)
$t_{1/2}$, hours	–	0.66 (0.54, 0.79)[a]	0.81 (0.61, 0.91)	0.90 (0.82, 1.07)	0.99 (0.92, 1.06)	1.05 (0.96, 1.21)
CL, mL/min		755 (28.4)[a]	700 (39.4)	661 (23.5)	863 (16.6)	719 (11.7)
V_z, L		45.1 (53.7)[a]	48.8 (54.0)	56.3 (42.3)	73.4 (16.4)	66.5 (17.2)
CL_r, mL/min			48.6 (17.7)	52.1 (26.4)	78.1 (23.6)	71.4 (25.9)
GS-441524						
AUC_{inf}, h·ng/mL	55.2 (27.6)[b]	264 (26.7)	1010 (31.1)	2470 (22.7)	4640 (16.2)	7350 (20.7)
AUC_{last}, h·ng/mL	19.3 (24.8)	181 (35.7)	893 (33.9)	2370 (23.2)	4520 (16.4)	7110 (20.9)
C_{max}, ng/mL	3.20 (10.9)	9.40 (28.4)	34.3 (30.9)	85.8 (23.9)	152 (23.6)	257 (30.2)
T_{max}, hours	5.00 (4.00, 5.00)	3.57 (3.00, 5.00)	4.00 (3.25, 4.00)	4.50 (3.50, 5.00)	4.00 (3.50, 4.00)	3.50 (3.00, 4.00)
$t_{1/2}$, hours	12.9 (7.35, 14.2)[b]	22.0 (19.8, 25.3)	27.3 (22.7, 29.6)	26.9 (25.4, 28.9)	27.4 (25.9, 28.8)	30.6 (29.5, 31.1)
CL_r, mL/min			116 (9.85)	117 (23.4)	127 (12.5)	136 (17.7)
GS-704277						
AUC_{inf}, h·ng/mL	11.1 (28.9)[c]	29.5 (20.9)	86.3 (32.8)	270 (49.0)	460 (19.7)	807 (19.8)
AUC_{last}, h·ng/mL	4.60 (54.1)	25.8 (24.4)	81.9 (33.7)	262.5 (49.9)	453 (20.0)	800 (19.8)
C_{max}, ng/mL	3.40 (21.1)	11.8 (22.6)	33.7 (31.4)	101 (57.9)	171 (22.3)	315.1 (19.5)
T_{max}, hours	2.25 (2.03, 2.25)	2.01 (2.00, 2.03)	2.02 (2.00, 2.03)	2.04 (2.03, 2.15)	1.98 (1.98, 2.18)	1.97 (1.96, 1.98)
$t_{1/2}$, hours	1.09 (0.72, 2.07)[c]	0.87 (0.85, 1.01)	1.09 (0.94, 1.18)	1.48 (1.29, 2.07)	1.81 (1.65, 1.91)	1.77 (1.39, 1.90)

Subjects received RDV solution i.v. formulation administered as a 2-hour infusion. All pharmacokinetic parameters are reported as mean (% coefficient of variation), except for T_{max} and $t_{1/2}$, which are reported as median (Q1, Q3). Plasma AUC_{inf} and $t_{1/2}$ were not estimable for RDV in cohort 1.
AUC_{inf}, area under the curve vs. time curve extrapolated to infinity; AUC_{last}, area under the curve from time zero to the last quantifiable concentration; CL, clearance; CL_r, renal clearance; C_{max}, peak plasma concentration; RDV, remdesivir; $t_{1/2}$, terminal half-life; T_{max}, time to peak concentration; V_z, volume of distribution. [a]Two for AUC_{inf}, $t_{1/2}$, V_z, CL for RDV in cohort 2. [b]Five for AUC_{inf}, $t_{1/2}$ for GS-441524. [c]Four for AUC_{inf}, $t_{1/2}$ for GS-704277 in cohort 1. Values presented to three significant figures.

Table 3 Pharmacokinetic parameters of RDV, GS-441524, and GS-704277 in cohorts 7–9 of the single-ascending-dose study

Parameter	Cohort 7 75 mg lyophilized formulation 2-hour infusion N = 10	Cohort 8 150 mg lyophilized formulation 2-hour infusion N = 10	Cohort 9 75 mg lyophilized formulation 30-minute infusion N = 9
RDV			
AUC_{inf}, h·ng/mL	1840 (17.1)	3260 (22.2)	1250 (19.6)
AUC_{last}, h·ng/mL	1830 (17.1)	3270 (21.3)	1250 (19.7)
C_{max}, ng/mL	1720 (28.4)	2720 (35.0)	2930 (29.2)
T_{max}, hours	2.00 (1.97–2.05)	1.99 (1.97–2.03)	0.50 (0.50–0.50)
$t_{1/2}$, hours	0.84 (0.80–0.96)	1.11 (0.97–1.80)	1.00 (0.85–1.03)
GS-441524			
AUC_{inf}, h·ng/mL	2200 (18.4)	4330 (22.2)	2020 (30.9)
AUC_{last}, h·ng/mL	2090 (19.0)	4190 (22.2)	1920 (32.8)
C_{max}, ng/mL	77.5 (21.0)	148 (26.5)	69.1 (32.8)
T_{max}, hours	3.25 (2.75–4.00)	4.00 (3.50–4.00)	3.50 (2.50–5.00)
$t_{1/2}$, hours	22.9 (21.7–27.0)	26.3 (24.2–28.7)	26.7 (25.0–26.9)
GS-704277			
AUC_{inf}, h·ng/mL	295 (27.9)	619 (24.6)	281 (39.0)
AUC_{last}, h·ng/mL	287 (28.3)	611 (24.9)	274 (40.0)
C_{max}, ng/mL	114 (25.7)	234 (28.8)	156 (43.0)
T_{max}, hours	2.02 (1.97–2.25)	1.99 (1.97–2.25)	0.50 (0.50–1.00)
$t_{1/2}$, hours	1.52 (1.43–1.76)	1.75 (1.36–1.85)	1.30 (1.23–1.44)

Subjects received RDV lyophilized i.v. formulation administered over a 2-hour infusion in cohorts 7 (75 mg) and 8 (150 mg) and over a 30-minute period in cohort 9 (75 mg). All pharmacokinetic parameters are reported as mean (% coefficient of variation), except for T_{max} and $t_{1/2}$, which are reported as median (Q1, Q3). One subject in cohort 9 did not receive the full volume of the intravenous dose; data for this subject were excluded. Values presented to three significant figures. AUC_{inf}, area under the curve vs. time curve extrapolated to infinity; AUC_{last}, area under the curve from time zero to the last quantifiable concentration; C_{max}, peak plasma concentration; RDV, remdesivir; $t_{1/2}$, terminal half-life; T_{max}, time to peak concentration.

Table 4 Summary statistics of PBMC pharmacokinetic parameters of GS-443902 in the single-ascending-dose study (cohorts 7–9)

GS-443902 PBMC PK Parameter	Cohort 7 RDV 75 mg lyophilized formulation 2 Hours (N = 10)	Cohort 8 RDV 150 mg lyophilized formulation 2 Hours (N = 10)	Cohort 9 RDV 75 mg lyophilized formulation 30 Minutes (N = 9)
AUC_{inf}, h*μM	176 (23.1)	297 (28.3)	394 (49.9)
AUC_{last}, h*μM	150 (19.7)	272 (28.8)	340 (47.4)
C_{max} μM	2.5 (16.2)	6.0 (46.1)	5.9 (37.7)
C_{24} μM	2.2 (23.3)	3.7 (40.9)	3.3 (55.7)
$t_{1/2}$, hour	42.7 (30.6–47.4)	36.0 (27.3–41.5)	49.0 (26.6–69.5)

PK parameters are reported as mean (% coefficient of variation); $t_{1/2}$ is reported as median (Q1, Q3). One subject in cohort 9 did not receive the full volume of the intravenous dose; data for this subject were excluded. Values presented to three significant figures.
AUC_{inf}, area under the curve vs. time curve extrapolated to infinity; AUC_{last}, area under the curve from time zero to the last quantifiable concentration; C_{max}, peak plasma concentration; PBMC, peripheral blood mononuclear cell; PK, pharmacokinetic; $t_{1/2}$, terminal half-life; T_{max}, time to peak concentration.

Table 5 Pharmacokinetic parameters of RDV, GS-441524, and GS-704277 in the multiple-dose study

PK parameters cohorts 1 and 2	Day 1 N = 16	Days 7 and 14 combined N = 23
RDV		
C_{max}, ng/mL	3170 (24.9)	3220 (20.0)
T_{max}, hours	1.03 (1.01, 1.05)	1.05 (1.03, 1.05)
$t_{1/2}$, hours	0.92 (0.80, 0.97)	1.03 (0.92, 1.09)
AUC,[a] h•ng/mL	2580 (20.1)	2700 (19.1)
CL_{ss}, mL/min	--	956 (17.5)
V_z, L	--	85.5 (22.8)
GS-441524		
C_{max}, ng/mL	139 (24.2)	231 (18.6)
T_{max}, hours	3.50 (2.50, 4.00)	3.00 (2.00, 4.00)
$t_{1/2}$, hours	22.0 (17.2, 26.9)	25.7 (23.3, 35.5)
AUC,[a] h•ng/mL	1950 (15.7)	3620 (15.1)
GS-704277		
C_{max}, ng/mL	282 (20.7)	435 (29.6)
T_{max}, hours	1.05 (1.03–1.06)	1.17 (1.05–1.17)
$t_{1/2}$, hours	1.58 (1.37–1.78)	1.80 (1.60–2.08)
AUC,[a] h•ng/mL	557 (21.3)	880 (31.8)

AUC, area under the curve vs. time curve; CL_{ss}, steady-state clearance; C_{max}, peak plasma concentration; RDV, remdesivir; $t_{1/2}$, terminal half-life; T_{max}, time to peak concentration; V_z, volume of distribution.
[a]AUC_{0-24} is presented for day 1, AUC_{tau} is presented for days 7 and 14 combined. All pharmacokinetic parameters are reported as mean (percent coefficient of variation), except for T_{max} and $t_{1/2}$, which are reported as median (Q1, Q3). Values presented to three significant figures.

AST elevation during the study. In the multiple-dose study, all AEs were low grade in severity and no serious AEs were reported. Elevations in ALT and AST were observed, with mild, reversible prothrombin time prolongation in some subjects but without other evidence of clinical hepatitis. The mechanism of these elevations is currently unknown.

Following single-dose i.v. administration of RDV solution over 2 hours, RDV, GS-704277, and GS-441524 exposures increased in a dose-proportional manner over the evaluated dose range (3 mg to 225 mg). Two higher doses evaluated in cohorts 1–6 (75 mg and 150 mg) were selected for evaluation in cohorts 7–9. This was designed to assess comparability of lyophilized formulation to solution formulation (cohort 7 and 8), as well as exploring shorter infusion duration (cohort 9). As expected, both the lyophilized and solution formulations demonstrated similar PK at single i.v. doses of 75 mg and 150 mg given over 2 hours indicating similar formulation performance. Additionally, the impact of a shorter 30-minute infusion duration on safety and PKs of RDV was also assessed using lyophilized formulation. A shorter infusion time was deemed beneficial in the setting of limited resources during COVID-19 outbreaks. Results demonstrated that sufficient concentrations of the active triphosphate metabolite, GS-443902, may be achieved following either a 2-hour or a 30-minute infusion; thereby, supporting recommendations of infusion times over 30 to 120 minutes in ongoing clinical studies. Following once-daily i.v. administration of 150 mg remdesivir for 7 or 14 days, RDV exhibited a PK profile similar to that observed during single-dose administration. Consistent with its short $t_{1/2}$ (~ 1 hour), RDV did not accumulate upon multiple dosing. Accumulation ratios (AUC and C_{max}) of 1.5-fold were noted for GS-704277, a finding that is not consistent with its short half-life (< 2 hours). Modest accumulation of < 2-fold was observed for the nucleoside metabolite GS-441524 ($t_{1/2}$ ~ 24.5 hours), reaching steady-state by day 4. Overall, data from our FIH studies in addition to nonclinical data formed the basis for the selection of the clinical regimen for the treatment of COVID-19 (single RDV 200 mg i.v. loading dose on day 1 followed by RDV 100 mg i.v. once daily maintenance doses from day 2 for a total treatment duration of either 5 days or 10 days).

Clinical trials to assess safety and efficacy of RDV for treatment of patients with COVID-19 are ongoing. Results are available from the two randomized clinical trials (the National Institute of Allergy and Infectious Diseases (NIAID) and GS-US-540-5773), a compassionate use program in patients with COVID-19, and from phase I clinical trials in healthy volunteers and subjects with Ebola viral disease.[19] Preliminary analysis of NIAID data showed a shorter median time to recovery (a metric used in influenza trials) and trends toward lower mortality rate in the RDV group (11 days and 8%) compared with placebo (15 days and 11.6%). The results from study GS-540-5773 suggested a similar improvement in clinical status in patients receiving a 10-day and a 5-day treatment courses of RDV. Based on available data, RDV was
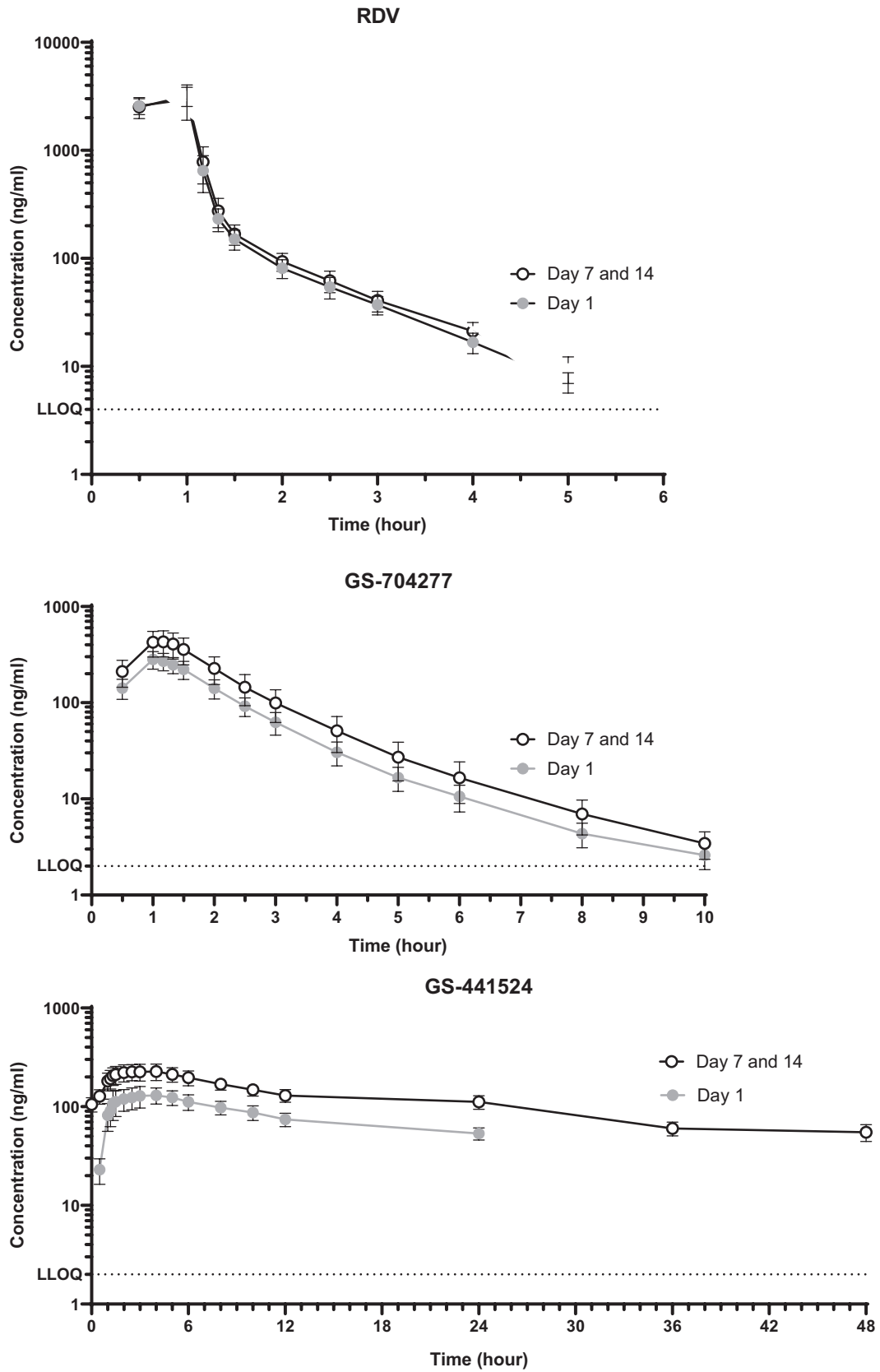
Figure 2 Plasma concentration-vs-time profiles following RDV multiple-dose administration; mean (±SD) values are plotted. LLOQ, lower limit of quantification; RDV, remdesivir.

granted an emergency use authorization for the treatment of COVID-19 in adults and children hospitalized with severe disease.[19] The availability of an effective antiviral agent, such as RDV, with a favorable benefit/risk profile would address a serious unmet medical need for the treatment of COVID-19 infected adults. Overall, the PK and safety data from the FIH study and preliminary clinical data supports continued investigation of RDV in patients with COVID-19.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www. cts-journal.com).

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