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REVIEW ARTICLE

Nucleoside analog GS-441524: pharmacokinetics in different species, safety, and potential effectiveness against Covid-19

Henrik Berg Rasmussen[1,2] | Ragnar Thomsen[3] | Peter Riis Hansen[4]

[1]Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Roskilde, Denmark

[2]Department of Science and Environment, Roskilde University Center, Roskilde, Denmark

[3]Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[4]Department of Cardiology, Herlev and Gentofte Hospital, Hellerup, Denmark

Correspondence
Henrik Berg Rasmussen, Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, DK-4000 Roskilde, Denmark.
Email: henrik.berg.rasmussen@regionh.dk

Abstract

GS-441524, the parent nucleoside of remdesivir, has been proposed to be effective against Covid-19 based on in vitro studies and studies in animals. However, randomized clinical trials of the agent to treat Covid-19 have not been conducted. Here, we evaluated GS-441524 for Covid-19 treatment based on studies reporting pharmacokinetic parameters of the agent in mice, rats, cats, dogs, monkeys, and the single individual in the first-in-human trial supplemented with information about its activity against severe acute respiratory syndrome coronavirus 2 and safety. A dosing interval of 8 h was considered clinically relevant and used to calculate steady-state plasma concentrations of GS-441524. These ranged from 0.27 to 234.41 μM, reflecting differences in species, doses, and administration routes. Fifty percent maximal inhibitory concentrations of GS-441524 against severe acute respiratory syndrome coronavirus 2 ranged from 0.08 μM to above 10 μM with a median of 0.87 μM whereas concentrations required to produce 90% of the maximal inhibition of the virus varied from 0.18 μM to more than 20 μM with a median of 1.42 μM in the collected data. Most of these concentrations were substantially lower than the calculated steady-state plasma concentrations of the agent. Plasma exposures to orally administered GS-441524, calculated after normalization of doses, were larger for dogs, mice, and rats than cynomolgus monkeys and humans, probably reflecting interspecies differences in oral uptake with reported oral bioavailabilities below 8.0% in cynomolgus monkeys and values as high as 92% in dogs. Reported oral bioavailabilities in rodents ranged from 12% to 57%. Using different presumptions, we estimated human oral bioavailability of GS-441524 at 13% and 20%. Importantly, doses of GS-441524 lower than the 13 mg/kg dose used in the first-in-human trial may be effective against Covid-19. Also, GS-441524 appears to be well-tolerated. In conclusion, GS-441524 has potential for oral treatment of Covid-19.

KEYWORDS
GS-441524, coronavirus disease 2019, in vitro–in vivo extrapolation, nucleoside analog, pharmacokinetics

Abbreviations: AUC_{0-24}, area under the plasma drug concentration–time curve from time zero to 24 h; AUC_{0-inf}, area under the plasma drug concentration–time curve from time zero to infinity; CC_{50}, 50 percent cytotoxic concentration; $C_{av,ss}$, average plasma drug concentration at steady state; Covid-19, coronavirus disease 2019; FIP, feline infectious peritonitis; HED, human equivalent dose; IC_{50}, 50 percent of maximal inhibitory concentration (half maximal inhibitory concentration); IC_{90}, 90 percent of maximal inhibitory concentration; PK, pharmacokinetics; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

1 | INTRODUCTION

There remains an unmet need for easily administrable therapeutic agents with high effectiveness in the treatment of coronavirus disease 2019 (Covid-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Drug repurposing has attracted interest to accelerate discovery and clinical development of new pharmacological Covid-19 treatments.[1] Remdesivir is a repurposed antiviral drug, which was originally designed to treat Ebola and other viral infections with a pandemic potential. This drug received approval for treatment of Covid-19 in USA and Europe based on the first stage of the Adaptive COVID-19 Treatment Trial known as ACTT-1.[2] However, subsequent results from the larger Solidarity trial questioned its effectiveness against Covid-19.[3] More recently, a systematic review and meta-analysis suggested that remdesivir accelerated recovery and increased rate of hospital discharge in subjects with Covid-19, but without significantly decreasing mortality or time to clinical improvement.[4]

Remdesivir belongs to the group of ProTides and has the adenosine analog GS-441524 as parent nucleoside.[5,6] Previously, GS-441524 was hypothesized to be superior to remdesivir for treatment of Covid-19.[7] Activity of GS-441524 against Covid-19 is supported by findings that it markedly inhibited SARS-CoV-2 in cell lines,[8] and possessed anti-SARS-CoV-2 activity in mouse models of Covid-19.[9] Also, GS-441524 has been found to be highly effective for treatment of feline infectious peritonitis (FIP), a coronavirus disease in cats.[10]

Since GS-441524 is a nucleoside analog lacking the McGuigan moiety characteristic of ProTides, its pharmacokinetics (PK) differs markedly from that of remdesivir. Notably, cellular uptake of GS-441524 is believed to be dependent upon membrane-bound transporters since it is hydrophilic with limited ability to cross cell membranes by diffusion.[11] By contrast, uptake of remdesivir appears to be mediated by diffusion, which is facilitated by its hydrophobic prodrug moieties, thus probably to a large extent being independent of membrane-bound transporters.[5,6,12] Once inside the cells, the GS-441524 is phosphorylated to the active antiviral GS-441524 triphosphate metabolite, also known as GS-443902, with adenosine kinase probably being responsible for catalyzing the first and perceived rate-limiting step in the formation of this metabolite.[13] Importantly, the relatively simple chemical structure of GS-441524 may permit fast manufacture of the agent in large amounts.[7]

Randomized clinical trials of GS-441524 against Covid-19 have not been conducted. Further knowledge about the pharmacokinetics, toxicity, and effectiveness against Covid-19 of GS-441524 may pave the way for the agent to reach such trials. Based on publicly available in vitro and in vivo data including data from a variety of different species, we here review the PK, anti-SARS-CoV-2 activity and safety of GS-441524 supplemented with calculations to critically evaluate the potential of this nucleoside analog for treatment of Covid-19.

2 | DATA SOURCES AND CALCULATIONS

PubMed, medRxiv and bioRxiv were searched on August 19, 2021 and revisited on December 19, 2021 using 'GS-441524' as search term to identify publications and preprints reporting on the PK, in vitro and in vivo anti-SARS-CoV inhibitory activities in addition to toxicity of the agent. PK data were also collected from the National Center for Advancing Translational Sciences.[14] Information about plasma protein binding of GS-441524 and PK parameters of GS-441524 triphosphate after administration of remdesivir in humans were obtained from recent publications[15] supplemented with reports assessing remdesivir for treatment of Covid-19 prepared by the European Medicines Agency[16] and the Australian Department of Health.[17]

In the event that a study presented plots of time versus plasma concentration for GS-441524 but did not provide PK variables, we extracted data from the plots using WebplotDigitizer (https://automeris.io/WebPlotDigitizer). Using the PKSolver, these data were used for calculation of PK parameters for a noncompartmental model including areas under the plasma drug concentration–time curve from time zero to 24 h (AUC_{0-24}) and from time zero to infinity (AUC_{0-inf}).[18] Conversions of animal doses to human equivalent doses (HEDs) and the yet only human dose to animal equivalent doses were based on allometric scaling with normalization to body surface area using the equation: HED = Animal dose x (Animal weight/Human weight)$^{(1-0.67)}$, where HED and animal dose are in mg/kg, animal and human weights are in kg and 0.67 is the body surface-based scaling factor.[19–21] Although this scaling factor is well-established for estimation of HEDs, 0.74 and 0.75 may perform better as factors for allometric-based interspecies drug dose conversion.[22,23] Hence, both 0.67 and 0.75 were used as scaling factor for estimation of human oral bioavailability of GS-441524.

3 | PLASMA PHARMACOKINETICS OF GS-441524 IN DIFFERENT SPECIES

GS-441524 doses and exposures following oral, intragastric, subcutaneous, intramuscular, and intravenous administration in mice, rats, cats, dogs, cynomolgus monkeys and a single human are listed (Table 1). Since the AUC_{0-24} and AUC_{0-inf} values were almost identical, GS-441524 does not appear to accumulate in plasma over a dosing interval of 24 h (data not shown). Therefore, we calculated average plasma concentrations of the agent at steady state ($C_{av,ss}$) using a dosing interval of 8 h and found that these concentrations ranged from 0.27 to 234.41 μM.

Assuming linear PK, the AUC_{0-inf} values in the animal species scaled to the only reported ($n = 1$) human dose of 13 mg/kg ranged from 20.23 μM · hour in cynomolgus monkey to 290,86 μM · hour in dogs (Table 2). Remarkably, the AUC_{0-inf} in cynomolgus monkey was closer to the value observed in the human of 31.08 μM · hour than it was to those reported in the other animal species.

Oral bioavailability of GS-441524 differed significantly between species, with cynomolgus monkeys displaying markedly lower ability

TABLE 1 Plasma pharmacokinetics of GS-441524 in various species

Species	Single dose route of administration	Dose (mg/kg)	Human equivalent dose (mg/kg)[a]	AUC_{0-inf} (uM·h)	$C_{av,ss}$ for three daily doses (uM)[b]	Reference
Cat	Subcutaneous	5	NA	41.26[c]	5.16	Murphy et al.[35]
Cat	Intravenous	5	2.05	42.42[c]	5.30	Murphy et al.[35]
Dog	Oral (capsule)	6.5	3.61	65.92	8.24	Yan et al.[24]
Rat	Intravenous	30	4.84	1875.28	234.41	Li et al.[9]
Rat	Intragastric	30	4.84	68.64	8.58	Li et al.[9]
Rat	Intravenous	5	0.81	11.07	1.38	NCATS[14]
Rat	Oral	10	1.61	7.47	0.93	NCATS[14]
Mouse	Intravenous	5	0.41	11.08	1.38	NCATS[14]
Mouse	Oral	10	0.81	8.71	1.09	NCATS[14]
Dog	Intravenous	2	1.11	28.73	3.59	NCATS[14]
Dog	Oral (solution)	5	2.78	65.50	8.19	NCATS[14]
Cynomolgus monkey	Intravenous	2	0.65	12.38	1.55	NCATS[14]
Cynomolgus monkey	Oral	5	1.61	2.51	0.31	NCATS[14]
Rat	Intramuscular	67	NA	634.88[c]	79.36	Shi et al.[49]
Mouse	Intramuscular	67	NA	357.81[c]	44.73	Shi et al.[49]
Mouse	Intravenous	10	0.81	30.40	3.80	Scherf-Clavel et al.[31]
Mouse	Intravenous	5	0.41	14.80	1.85	Xie and Wang[32]
Mouse	Oral	10	0.81	16.84	2.10	Xie and Wang[32]
Rat	Oral	10	1.61	2.14[c]	0.27	Yin et al.[50]
Rat	Intravenous	2	0.32	2.49[c]	0.31	Yin et al.[50]
Human	Oral, fasted	13	13	31.08[d]	3.89	Yan[34]

Note: NCATS: The National Center for Advancing Translational Sciences; AUC_{0-inf}: area under the plasma drug concentration–time curve from time zero to infinity; $C_{av,ss}$: average plasma drug concentration at steady state; NA: not applicable.

[a]Animal doses were converted to human equivalent doses using the exponent 0.67 in body surface area-based allometric scaling. Since interspecies conversion of drug doses by allometric scaling is not supported for subcutaneous and intramuscular administration, only human equivalent doses for oral and intravenous administrations were calculated.

[b]Calculated as AUC_{0-inf}/τ, where τ is the doing interval (8 h).

[c]For studies that presented plots of time versus drug concentrations without providing AUC_{0-inf} values, these values were calculated using PKSolver after extraction of data from the plots. Moreover, we recalculated areas under the plasma drug concentration–time curve from time zero to 12 or 24 h based on data extracted from plots and found that these did not deviate with more than 5% from the corresponding parameters of exposure reported by the studies in question (data not shown).

[d]Calculated based on the supplementary data appended the first-in-human study ($n = 1$).

for oral uptake than mice and dogs (Table 3). Human oral bioavailability of GS-441524 was estimated at 13% and 20% using the scaling factors of 0.67 and 0.75, respectively. These levels of human bioavailability are consistent with previous estimates ranging from 15% to 30% obtained by comparison with acyclovir.[24] Hence, the human oral bioavailabilities calculated using different approaches suggest that oral administration of GS-441524 is feasible in humans.

4 | IN VITRO ANTI-SARS COV-2 ACTIVITY OF GS-441524

Using different isolates of SARS-CoV-2, viral quantification methods and types of cells, studies have reported fifty percent maximal inhibitory concentration (IC_{50}) values from 0.08 to above 10 μM with a median of 0.87 μM and 90% maximal inhibitory concentration (IC_{90}) values from 0.18 to above 20 μM corresponding to a median of 1.42 μM for the activity of GS-441524 against the virus (Table 4). Most of the calculated $C_{av,ss}$ values exceeded these medians (Table 1). A study showed that GS-441524 at 3.7 μM reduced the load of SARS-CoV-2 RNA in Vero cells by more than four log_{10} units to levels below lower limit of detection but did not determine the IC_{50} and IC_{90} values for its activity against the virus.[25] Consistent with this, exposure to GS-441524 at a concentration of 3 μM reduced the load of SARS-CoV-2 with up to four log_{10} units in cultured human airway epithelial cells, which may represent a more appropriate model for studying the activity of anti-SARS-CoV-2 agents than cancer cell lines.[26] Other findings, also based on human airway epithelial cell

TABLE 2 Area under the plasma drug concentration–time curve after oral administration of GS-441524 adjusted to human dose in different animal species

Species	Administered dose (mg/kg)	Observed AUC_{0-inf} (uM · h)[a]	Scaled AUC_{0-inf} (uM · h)[b]	Reference[c]
Mouse	10	8.71	139.27	NCATS[14]
Rat	10	7.47	60.21	NCATS[14]
Cynomolgus monkey	5	2.51	20.23	NCATS[14]
Dog	5	65.50	306.54	NCATS[14]
Human[d]	13	31.08	NA	Yan 2021[34]

Note: NA, not applicable; NCATS, The National Center for Advancing Translational Sciences; AUC_{0-inf}, area under the plasma drug concentrationa curve from time zero to infinity.

[a]Determined using PKSolver.

[b]Animal AUC_{0-inf} values scaled to a human dose of 13 mg/kg under the assumption of dose-related pharmacokinetics. These AUC_{0-inf} values were calculated by multiplying observed animal AUC_{0-inf} values with the ratio between the animal equivalent dose and the administered animal dose, where the animal equivalent doses were derived by body surface-based allometric scaling of the human dose.

[c]To increase comparability, animal data from NCATS only were used.

[d]First-in-human study ($n = 1$).

TABLE 3 Oral bioavailability of GS-441524 in different species

Species	Oral bioavailability (%)[a]	Reference
Mouse	39	NCATS[14]
Mouse	57	Xie and Wang[32]
Rat	12	Mackman et al.[51]
Rat	16	Yin et al.[50]
Rat	33	NCATS[14]
Dog	85	NCATS[14]
Dog	89	Mackman et al.[51]
Dog	92	Yan et al.[24]
Cynomolgus monkey	3[b]	Mackman et al.[51]
Cynomolgus monkey	8	NCATS[14]
Human	13[c]	Present review

Note: NCATS, The National Center for Advancing Translational Sciences.

[a]Oral administration formulation was a solution.

[b]Value estimated at 3.4%, which we rounded to 3%.

[c]Calculated using the equation: Oral bioavailability = 100 · AUC_{Oral}/AUC_{IV} · $Dose_{IV}$/$Dose_{Oral}$, where AUC_{Oral} and AUC_{IV} are the areas under the plasma drug concentration–time curves after administration of $Dose_{Oral}$ and $Dose_{IV}$, respectively. A human AUC_{oral} of 31.08 uM·h measured after administration of an oral dose of 13 mg/kg was used for the calculation. Given that the AUC_{IV} of GS-441524 has not been determined in humans, this plasma exposure in the equation was replaced with the AUC_{IV} of 12.38 uM·h reported in cynomolgus monkeys after administration of 2 mg/kg. Furthermore, a human equivalent intravenous dose of 0.65 mg/kg, which we obtained by body surface-based allometric scaling of the monkey intravenous dose of 2 mg/kg (scaling factor = 0.67), served as intravenous dose, thus assuming that the scaled dose produces an AUC_{iv} in humans similar to that observed in cynomolgus monkeys. Using a scaling factor of 0.75, human oral bioavailability of GS-441524 was estimated at 20%.

cultures, found that GS-441524 at a concentration of 2 μM resulted in complete elimination of SARS-CoV-2, whereas 1 μM of the agent conferred intermediate anti-SARS-CoV-2 activity.[27]

Importantly, plasma protein binding of GS-441524 is low with the unbound fraction being above 85%[16] or even as high as 98% to 99%.[17] Hence, a major fraction of GS-441524 in human plasma appears to be available for cellular uptake and subsequent intracellular phosphorylation to the active antiviral GS-441524 triphosphate metabolite.

Based on detection of fine changes in cellular morphology, studies have reported that GS-441524 potently rescued the phenomic profile induced by SARS-CoV-2 and strongly suppressed alterations in this profiles, thus suggesting activity against the virus.[28,29] By contrast, findings based on a cellular thermal shift assay coupled to mass spectrometry, did not show anti-SARS-CoV-2 activity of GS-441524 in HepG2 cells, possibly reflecting limited cellular uptake of the agent.[30] However, these analytical procedures were designed for high-throughput screening and may therefore not provide accurate information about the anti-SARS-CoV activity of all agents.

5 | TISSUE DISTRIBUTION AND IN VIVO ANTI-SARS-COV-2 ACTIVITY OF GS-441524

A single intravenous injection of mice with GS-441524 at a dose of 10 mg/kg has been reported to produce wet weight concentrations above 1 μmol/kg in most organs, except for lungs and nasal mucosa, leading to the suggestion that administration of the agent at 10 mg/kg twice daily in mice would produce concentrations above reported IC_{50} values for its anti-SARS-CoV-2 activity in most organs.[31] In support of this suggestion, a plot of time versus concentration showed that administration of a single oral dose of 20 mg/kg GS-441524 to mice resulted in concentrations above 2 μmol/kg in the lungs and up to 10 μmol/kg in the liver at 1, 2 and 4 h post dosing.[32] Also, a daily intraperitoneal GS-441524 dose of 25 mg/kg resulted in significant inhibition of SARS-Cov-2 in a mouse model of Covid-19 after 2 days of treatment.[9] Given that the intracellular volume in many tissues is about 0.8 mL/g tissue,[33] intracellular concentrations of GS-441524 in μM are likely to be 25% higher than the reported wet weight

TABLE 4 Anti-SARS-CoV-2 activity of GS-441524

SARS-CoV-2 strain or isolate[a]	Cell line or primary cell culture	Virus quantification method	IC_{50} (μM)	IC_{90} (μM)	Reference
hCoV-19/CHN/SYSU-IHV/2020	Vero E6	RT-qPCR	0.70	-	Li et al.[9]
hCoV-19/CHN/SYSU-IHV/2020	Calu-3	RT-qPCR	3.21	-	Li et al.[9]
hCoV-19/CHNg/SYSU-IHV/2020	Caco-2	RT-qPCR	3.62	-	Li et al.[9]
hCoV-19/USA/WA1/2020	Vero E6	Plaque forming assay	0.47	0.71	Pruijssers et al.[8]
hCoV-19/USA/WA1/2020	Vero E6	RT-qPCR	0.47	0.80	Pruijssers et al.[8]
hCoV-19/USA/WA1/2020	Calu3 2B4	Plaque forming assay	0.62	1.34	Pruijssers et al.[8]
hCoV-19/USA/WA1/2020	Calu3 2B4	RT-qPCR	1.09	1.37	Pruijssers et al.[8]
hCoV-19/Harbin/HRB-26/2020	Vero E6	Plaque forming assay	5.19	-	Shi et al.[49]
hCoV-19/mouse/Harbin/HRB-26m/2020 (mouse-adapted)	Vero E6	Plaque forming assay	5.05	-	Shi et al.[49]
hCoV-19/USA/WA1/2020 with insertion of nanoluciferase gene	A549 expressing human angiotensin-converting enzyme 2	Luciferase signal	0.87	-	Xie et al.[52]
hCoV-19/Wuhan/WIV04/2019	Vero E6	RT-qPCR	0.48	-	Yin et al.[50]
hCoV-19/USA/WA1/2020 and B.1.351 (beta) isolate	Calu-3	Counting infected cells using fluorescence microscopy	0.10	-	Schultz et al.[53]
hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein	293T	Counting cells expressing reporter or measuring luciferase activity	0.60	-	He et al.[54]
hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein	Vero	Counting cells expressing reporter or measuring luciferase activity	0.30	-	He et al.[54]
hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein	Huh-7.5	Counting cells expressing reporter or measuring luciferase activity	1.03	-	He et al.[54]
hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein	Calu-1	Counting cells expressing reporter or measuring luciferase activity	1.33	-	He et al.[54]
hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein	A549	Counting cells expressing reporter or measuring luciferase activity	1.47	-	He et al.[54]
hCoV-19/Belgium/GHB-03021/2020	Vero E6 expressing enhanced green fluorescent protein	Fluorescence intensity measurement	2.74[b]	-	Saul et al.[55]
hCoV-19/Belgium/GHB-03021/2020	Vero E6 expressing green fluorescent protein	Fluorescence-based imaging	0.78–0.89[c]	-	Do et al.[27]
hCoV-19/Belgium/GHB-03021/2020	Huh-7	Cytopathic effect	1.10–1.50[c]	-	Do et al.[27]
hCoV-19/Belgium/GHB-03021/2020 and hCoV-19/Germany/BY-ChVir-929/2020	Human airway epithelial cells	RT-qPCR	0.51	-	Do et al.[27]
hCoV-19/USA/WA1/2020	Vero	RT-qPCR	8.2	13.2	Zandi et al.[56]
hCoV-19/USA/WA1/2020	Huh-7	RT-qPCR	>10	>20	Zandi et al.[56]
hCoV-19/CHN/SYSU-IHV/2020	Vero E6	RT-qPCR	1.71	-	Cao et al.[57]
SARS_CoV-2_human_CHN_20SF18530_2020	Vero E6	RT-qPCR	1.35	-	Cao et al.[57]
B.1.617.2 isolate	Vero E6	RT-qPCR	0.96	-	Cao et al.[57]
hCoV-19/Wuhan/WIV04/2019	Vero E6	RT-qPCR	0.48	-	Wei et al.[58]
hCoV-19/USA/WA1/2020 expressing nanoluciferase	A549 expressing human angiotensin-converting enzyme 2	Luminescence intensity measurement	3.37	-	Schäfer et al.[59]
hCoV-19/USA/WA1/2020 expressing firefly luciferase	Normal human bronchial epithelial cells	Luminescence intensity measurement	2.45	-	Schäfer et al.[59]

(Continues)

TABLE 4 (Continued)

SARS-CoV-2 strain or isolate[a]	Cell line or primary cell culture	Virus quantification method	IC_{50} (μM)	IC_{90} (μM)	Reference
hCoV-19/USA/WA1/2020 expressing mNeonGreen protein	Vero E6	Fluorescent-reporter imaging to quantitate focus-forming units	0.42	0.60	Lo et al.[60]
hCoV-19/USA/WA1/2020 expressing mNeonGreen protein	Huh-7	Fluorescent-reporter imaging to quantitate focus-forming units	0.69	1.50	Lo et al.[60]
hCoV-19/USA/WA1/2020	Vero E6	RT-qPCR	0.38	0.77	Schooley et al.[61]
hCoV-19/USA/WA1/2020	Human pluripotent stem cell-derived lung cells	RT-qPCR	0.74	2.62	Schooley et al.[61]
hCoV-19/USA/WA1/2020	Calu-3	RT-qPCR	0.15	0.18	Schooley et al.[61]
hCoV-19/USA/WA1/2020	Huh-7.5	RT-qPCR	0.32	0.73	Schooley et al.[61]
hCoV-19/USA/WA1/2020	Caco-2	RT-qPCR	0.96	1.75	Schooley et al.[61]
hCoV-19/USA/WA1/2020	Vero E6	RT-qPCR	1.10	3.90	Tao et al.[62]
hCoV-19/USA/WA1/2020	Vero	RT-qPCR	0.80	1.60	Tao et al.[62]
hCoV-19/USA/WA1/2020	Calu-3	RT-qPCR	0.25	2.35	Tao et al.[62]
hCoV-19/USA/WA1/2020	Caco-2	RT-qPCR	0.08	1.42	Tao et al.[62]
hCoV-19/USA/WA1/2020	Caco-2	Fluorescent-reporter imaging to quantitate focus-forming units	1.30	-	Tao et al.[62]

Note: Origin of cell lines: Vero and Vero E6, African green monkey kidney; Calu-1, Calu-3 and Calu3 2B4, human metastatic lung adenocarcinoma; Caco-2, human colorectal adenocarcinoma; A549, human epithelial lung carcinoma; 293T, human embryonic kidney; Huh-7 and Huh-7.5, human hepatocellular carcinoma; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IC_{50}, 50 percent of maximal inhibitory concentration (half maximal inhibitory concentration); IC_{90}, 90 percent of maximal inhibitory concentration; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction.

[a]Isolate designation is based on GISAID (https://www.gisaid.org/) when possible. Isolate hCoV-19/USA/WA1/2020 is now designated hCoV-19/USA/WA-CDC-02982586-001/2020, and hCoV-19/CHN/SYSU-IHV/2020 is now known as hCoV-19/Guangdong/SYSU-IHV/2020.

[b]Value estimated based on a plot.

[c]Interquartile range (Q1–Q3).

concentrations.[31,32] Assuming such ratio between volume and weight, we derived intracellular concentrations of GS-441524 from 1.25 to above 10 μM in mice after a single GS-441524 dose of 10 mg/kg intravenously or 20 mg/kg orally,[31,32] i.e. equal to or higher than the median IC_{50} and IC_{90} values of 0.87 and 1.42 μM for the anti-SARS-CoV-2 activity of the agent with its concentration in the liver being more than 7-fold higher than the IC_{90} median value (Table 4). Also, the intracellular concentrations of GS-441524 exceeded the IC_{50} and IC_{90} values by more than 4-fold in a large number of other organs.[31]

We calculated HEDs of 1.63, 1.63 and 2.03 mg/kg/day by body surface-based scaling of the GS-441524 dose of 10 mg/kg suggested to be administered intravenously twice a day,[31] the single peroral dose of 20 mg/kg[32] and the daily intraperitoneal injection of 25 mg/kg,[9] which all possessed potential for inhibition of SARS-CoV-2 in mice. These HEDs were considerably lower than the 13 mg/kg dose administered once or three times daily in the first-in-human study.[34]

6 | CELLULAR PHARMACOKINETICS OF GS-441524 TRIPHOSPHATE

A study reported intracellular GS-441525 triphosphate levels from 1 to 1.25 μM in Crandell-Rees feline kidney cells exposed to GS-441524 at 1 μM over a three-day period.[35] Moreover, intracellular levels of GS-441524 triphosphate of 0.85 and 1.78 pmol/million cells were reported in Calu3 2B4 cells and Vero E6 cells, respectively, after incubation with GS-441524 for 24 h.[8] Assuming an intracellular average volume of 1 pL in cultured mammalian cells,[35] these levels translate into intracellular concentrations of 0.85 and 1.78 μM. Based on intracellular volumes determined specifically for Calu-3 cells of 2.7 pL/cell[36] and Vero cells ranging from 0.59 to 0.74 pL/cell,[37] the intracellular concentrations of GS-441524 triphosphate were estimated at 0.31 μM in Calu cells and from 2.41 to 3.02 μM in Vero E6 cells suggesting major differences between different cell types in uptake and ability to phosphorylate adenosine analogs.

Also, large variation in concentrations of GS-441524 triphosphate between organs after administration of GS-441524 have been reported. Notably, a plot of time versus concentration showed that administration of a single oral GS-441524 dose of 20 mg/kg to mice led to concentrations in the range from about 0.20 to 0.63 μmol/kg and from 0.04 to 0.10 μM μmol/kg in homogenated liver and lung, respectively, 1–4 h after dosing.[32] The GS-441524 triphosphate concentrations observed in lung homogenate translate into intracellular concentrations in the range from 0.05 to 0.13 μM assuming an intracellular volume in the lung of 0.8 mL/g tissue.[33] Since conversion of a human dose of GS-441524 at 13 mg/kg by

body surface-based interspecies scaling yields a mouse equivalent dose of 160 mg/kg, i.e. eight times higher than the 20 mg/kg dose,[32] a single dose of 160 mg/kg is expected to produce intracellular lung concentrations of GS-441524 triphosphate from 0.40 to 1.00 μM in mice under the assumption of linear PK and validity of interspecies extrapolation.

Using data extracted from a plot of time versus concentration[35] followed by calculation of AUC_{0-24}, we derived intracellular GS-441524 triphosphate average concentrations of 8 and 17 μM over a period of 24 h in peripheral blood mononuclear cells from cats after a single intravenous or subcutaneous administration of GS-441524 at 5 mg/kg, respectively, corresponding to a HED of 2.16 mg/kg. For comparison, remdesivir administered intravenously to healthy human subjects at therapeutic doses, i.e. 100 mg daily after an initiation dose of 200 mg, has been found to produce a steady-state AUC of GS-441524 triphosphate of 240 h · μM in peripheral blood mononuclear cells.[15] This translates into a $C_{av,ss}$ of 10 μM over a 24-h dosing interval, which is almost equal to or lower than the estimated GS-441524 triphosphate concentrations of 9 and 17 μM in peripheral blood mononuclear cells from cats administered a single GS-441524 dose of 5 mg/kg.[35] A lower average GS-441524 triphosphate concentration of 6.54 μM in peripheral blood mononuclear cells was calculated for the first 24 h following a remdesivir initiation dose of 200 mg/kg in humans.[15]

We derived IC_{50} values for the intracellular anti-SARS-CoV-2 activity of GS-441524 triphosphate by multiplication of the ratios between intracellular GS-441524 triphosphate and extracellular GS-441524 levels with the IC_{50} values for the anti-SARS-CoV-2 activities of extracellular GS-441524 in Calu3 2B4 and Vero E6 cells, respectively, based on cell-specific intracellular volumes.[8] Using this approach, we obtained an IC_{50} value of approximately 0.27 μM in Calu3 2B4 cells and IC_{50} values from 1.13 to 1.42 μM in Vero E6 cells. These intracellular IC_{50} values are markedly higher than the intracellular concentrations of GS-441524 triphosphate ranging from 0.05 to 0.13 μM in mouse lungs but only slightly higher than the intracellular lung concentrations in mice estimated assuming administration of a mouse dose equivalent with the human dose of 13 mg/kg.[32,34] Also, the IC_{50} values for the intracellular anti-SARS-CoV-2 activity of GS-441524 triphosphate were comparable to the observed concentrations of this compound in mouse liver after conversion of wet weight concentrations to intracellular concentrations,[32] but significantly lower than the intracellular GS-441524 triphosphate concentration in peripheral blood cells from cats administered GS-441524 and humans administered remdesivir.[15,35] Notably, the IC_{50} value of 0.27 μM was almost 63-fold lower than the GS-441524 triphosphate concentration of 17 μM in cat peripheral blood mononuclear cells.[35] Therefore, GS-441524 triphosphate seems to be formed at sufficiently high intracellular levels after administration of GS-441524 and remdesivir in several types of cells to inhibit SARS-CoV-2 with the level of this active metabolite probably being lower in the lungs than in most other organs.

7 | SAFETY AND TOXICITY

Reported values for the 50% cytotoxic concentration (CC_{50}) of GS-441524 ranged from 7 to above 1000 μM suggesting low generalin vitrocytotoxicity of the agent (Table 5). In line with this, the recommendations on compassionate use of remdesivir by the European Medicines Agency concluded that high levels of GS-441524 did not induce in vitro cytotoxic effects, although CC_{50} values in the range from 9.6 to 13.9 μM affected hematopoietic stem cell proliferation.[16] However, these concentrations are 3 to 4.5-fold higher than the C_{max} of 3.05 μM observed after administration of a single dose of GS-441524 at 13 mg/kg in the first-in-human study.[34] Moreover, GS-441524 at a concentration as high as 50 μM was required to produce alteration in the in vitro antigen-induced memory T cell proliferation.[38] Consistent with this, concentrations of GS-441524 in the range between 10 and 100 μM were necessary to inhibit proliferation of NRK-49F cells and HK-2 cells, derived from rat and human kidneys, after stimulation with transforming growth factor-β, whereas marked inhibition of the protein expression of fibrotic markers such as fibronectin was detected at a concentration of this nucleoside analog of 10 μM.[39]

In line with the in vitro findings, preclinical animal studies have suggested that GS-441524 is devoid of adverse effects at high doses. This includes multiple oral doses of 150 mg/kg in mice and 20 mg/kg in non-human primates.[34] Additionally, a dose range-finding study suggested that GS-441524 was well-tolerated at maximum feasible oral doses of 1,000, 1,500 and 2,000 mg/kg/day in cynomolgus monkeys, rats and dogs, respectively.[14]

A clinical trial with 31 cats suffering from FIP found no systemic toxicity after treatment with doses of GS-441524 at 2 and 4 mg/kg for 12 to 30 weeks.[10] Moreover, administration of GS-441524 at doses of 5, 8 and 10 mg/kg for up to 19 weeks did not produce major adverse reactions in four cats with FIP.[40] The therapeutic doses of 2, 4, 5, and 10 mg/kg, which appeared to be well-tolerated in cats, correspond to HEDs in the range from 0.82 to 4.10 mg/kg per day. These HEDs are significantly lower than the GS-441524 dose of 13 mg/kg that was administered to a healthy human volunteer once daily for seven days and three times daily for three days, respectively, and reported to not be associated with major adverse reactions or significant alterations of key blood parameters.[34] Hence, the current evidence suggests that GS-441524 is safe in a range of species, albeit with a scarcity of human data being available at present.

8 | EFFECTS OF EXTRACELLULAR AND INTRACELLULAR ADENOSINE LEVELS ON THERAPEUTIC EFFECTIVENESS

Previously, we suggested that endogenous adenosine competes with GS-441524 for cellular uptake by nucleoside transporters and the perceived rate-limiting first step of its phosphorylation to GS-441524 monophosphate eventually leading to the formation of GS-441524 triphosphate.[11] Under physiological conditions,

TABLE 5 Cytotoxicity of GS-441524

Cell line or primary cell culture	CC_{50} (μM)[a]	Reference
Vero E6	>50	Li et al.[9]
Calu-3	>50	Li et al.[9]
Caco-2	>50	Li et al.[9]
Vero E6	>250	Shi et al.[49]
A549 expressing human angiotensin-converting enzyme 2	>50	Xie et al.[52]
Vero E6	>1000	Yin et al.[50]
293T	>30[b]	He et al.[54]
VeroE6 tagged green fluorescent protein	49–83[c]	Do et al.[27]
Huh-7	37–59[c]	Do et al.[27]
Calu-3	>50	Schulz et al.[53]
Human peripheral blood mononuclear cells	>100	Zandi et al.[56]
CEM	>100	Zandi et al.[56]
Vero	>100	Zandi et al.[56]
Huh-7	>100	Zandi et al.[56]
CRFK	>100	Cook et al.[63]
CRFK	>100	Murphy et al.[35]
NRK-49F stimulated with transforming growth factor-β	>100	Xu et al.[39]
Vero E6	>50	Cao et al.[57]
HEL	>100	Stevaert et al.[64]
Vero E6	>100	Lo et al.[60]
Huh-7	>100	Lo et al.[60]
HSAEC1-KT	>100	Lo et al.[60]
TIME	>100	Lo et al.[60]
Vero E6	>100	Schooley et al.[61]
Human pluripotent stem cell-derived lung cells	>100	Schooley et al.[61]
Calu-3	>100	Schooley et al.[61]
Huh-7.5	>100	Schooley et al.[61]
Caco-2	>100	Schooley et al.[61]
Vero CCL-81	>100	Tao et al.[62]
Calu-3	>100	Tao et al.[62]
Caco-2	>100	Tao et al.[62]
Huh-7	>100	Tao et al.[62]

Note: Origin of cell lines: Vero and Vero E6, African green monkey kidney; Calu-3, human metastatic lung adenocarcinoma; Caco-2, human colorectal adenocarcinoma; A549, human epithelial lung carcinoma; 293T, human embryonic kidney; Huh-7 and Huh-7.5, human hepatocellular carcinoma; CEM, human acute lymphoblastic leukemia; CRFK, cat kidney cortex; NRK-49F, normal rat kidney (fibroblasts); HEL, human erythroleukemia; HSAEC1-KT, human small airway epithelial cells (human telomerase reverse transcriptase-immortalized); TIME, telomerase-immortalized human microvascular endothelium; CC_{50}, 50 percent cytotoxic concentration.

[a]Several studies reported upper concentrations of serial dilutions of GS-441524 for which cell toxicity was not observed but did not determine CC_{50} values. In such instances, these upper tested concentrations were used as substitutes for CC_{50} values.

[b]Determined based on a plot.

[c]Interquartile range (Q1–Q3).

extracellular and intracellular adenosine levels are in the submicromolar range, but these levels are significantly elevated in hypoxia and critical illness, occasionally being increased by 5- to 10-fold or more, e.g. reaching a level of 8.4 μM in sepsis.[41–44] Such high adenosine levels exceed most of the calculated $C_{av,ss}$ values of GS-441524 including that of 3.89 μM reported in the first-in-human study (Table 1). Importantly, high blood levels of uridine were recently suggested to competitively inhibit cellular uptake of EIDD-1931 (https://www.guidetopharmacology.org/GRAC/DatabaseSearchForward?searchString=EIDD-1931&searchCategories=all&species=none&type=all&comments=includeComments&order=rank), the main metabolite of the cytosine analog prodrug molnupiravir (https://www.guidetopharmacology.org/GRAC/DatabaseSearchForward?searchString=molnupiravir&searchCategories=all&species=none&type=all&comments=includeComments&order=rank&submit=Search+GtoPdb) with activity against SARS-CoV-2,[45] thus supporting the notion that levels of endogenous nucleotides can affect therapeutic effectiveness of nucleoside analogs.

9 | CONCLUSION

In aggregate, publicly available data suggest that GS-441524 has potential for oral treatment of Covid-19 although human oral bioavailability does not appear to be high. Of note, a lower oral dose than the 13 mg/kg dose administered in the so far only reported human PK and safety study ($n = 1$) may be effective against Covid-19. However, GS-441524 may not be equally effective in eliminating SARS-CoV-2 from all tissues and organs, potentially having lower activity against the virus in the airways than in other organs. Also, cellular uptake and intracellular phosphorylation of GS-441524 necessary for antiviral activity could be reduced by competitive inhibition due to increased adenosine levels in subjects with severe Covid-19. GS-441524 appears to be well tolerated in animal species, and although to our knowledge, new clinical studies are not currently registered in major clinical trial registries, further clinical development of the agent appears to be justified.

9.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[46] and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.[47,48]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Henrik Berg Rasmussen identified and collected relevant findings from publicly available sources, conducted calculations, and drafted

the article. Peter Riis Hansen co-wrote the article and provided critical revision. Ragnar Thomsen provided critical revision and suggestions. All three authors interpreted the results from the calculations and approved the final manuscript.

DATA AVAILABILITY STATEMENT

No data available in the study.

ORCID

Henrik Berg Rasmussen https://orcid.org/0000-0001-9979-7627

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