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Antiviral Research 198 (2022) 105246

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Subcutaneous remdesivir administration prevents interstitial pneumonia in rhesus macaques inoculated with SARS-CoV-2

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ARTICLE INFO

Keywords:
COVID-19
Antiviral
Animal model

ABSTRACT

The utility of remdesivir treatment in COVID-19 patients is currently limited by the necessity to administer this antiviral intravenously, which has generally limited its use to hospitalized patients. Here, we tested a novel, subcutaneous formulation of remdesivir in the rhesus macaque model of SARS-CoV-2 infection that was previously used to establish the efficacy of remdesivir against this virus in vivo. Compared to vehicle-treated animals, macaques treated with subcutaneous remdesivir from 12 h through 6 days post inoculation showed reduced signs of respiratory disease, a reduction of virus replication in the lower respiratory tract, and an absence of interstitial pneumonia. Thus, early subcutaneous administration of remdesivir can protect from lower respiratory tract disease caused by SARS-CoV-2.

The nucleotide prodrug remdesivir was shown to be effective against SARS-CoV-2 in rhesus macaques early after the emergence of this virus (Williamson et al., 2020). The first clinical trial data indicated a shorter time to recovery in hospitalized COVID-19 patients treated with remdesivir (Beigel et al., 2020). However, mixed results were obtained in subsequent clinical trials assessing the efficacy of remdesivir in COVID-19 patients (reviewed in (Aleissa et al., 2020)). The use of remdesivir as a treatment for COVID-19 has largely been limited to hospitalized patients whose disease has already progressed due to the fact that the current formulation of remdesivir has to be administered intravenously on 5 consecutive days. However, it was shown early on that treatment earlier during infection when virus replication peaks, would likely result in a greater treatment efficacy (Beigel et al., 2020). Indeed, an 87% reduction in hospitalization in non-hospitalized, high-risk COVID-19 patients was recently observed in a randomized, placebo-controlled clinical trial assessing outpatient treatment with a 3-day course of remdesivir (Gottlieb et al., 2021). Alternate routes of administration such as subcutaneous injection may enable earlier treatment prior to hospitalization which could maximize the therapeutic benefit of remdesivir.

A comparison of the pharmacokinetics of the subcutaneous remdesivir formulation to the previously used intravenous formulation was performed in healthy, uninfected Indian-origin rhesus macaques. Levels of remdesivir and its main metabolites were measured by LC-MS/MS in plasma and lung tissue as previously described (Cox et al., 2021). As expected, somewhat different pharmacokinetic profiles for remdesivir and its metabolites were observed following subcutaneous administration (Table S1 and Fig. S1). Compared to intravenous administration, 30-fold and 8-fold lower maximal concentrations (C_{max}), with more persistent levels, were observed for remdesivir (GS-5734) and its alanine metabolite GS-704277, respectively (Table S1). Additionally, the nucleoside metabolite GS-441524 appeared more slowly in plasma and persisted with a longer estimated terminal elimination half-life following subcutaneous dosing. Daily exposures (AUC_{0-24h}) were

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https://doi.org/10.1016/j.antiviral.2022.105246
Received 2 November 2021; Received in revised form 30 December 2021; Accepted 10 January 2022
Available online 13 January 2022

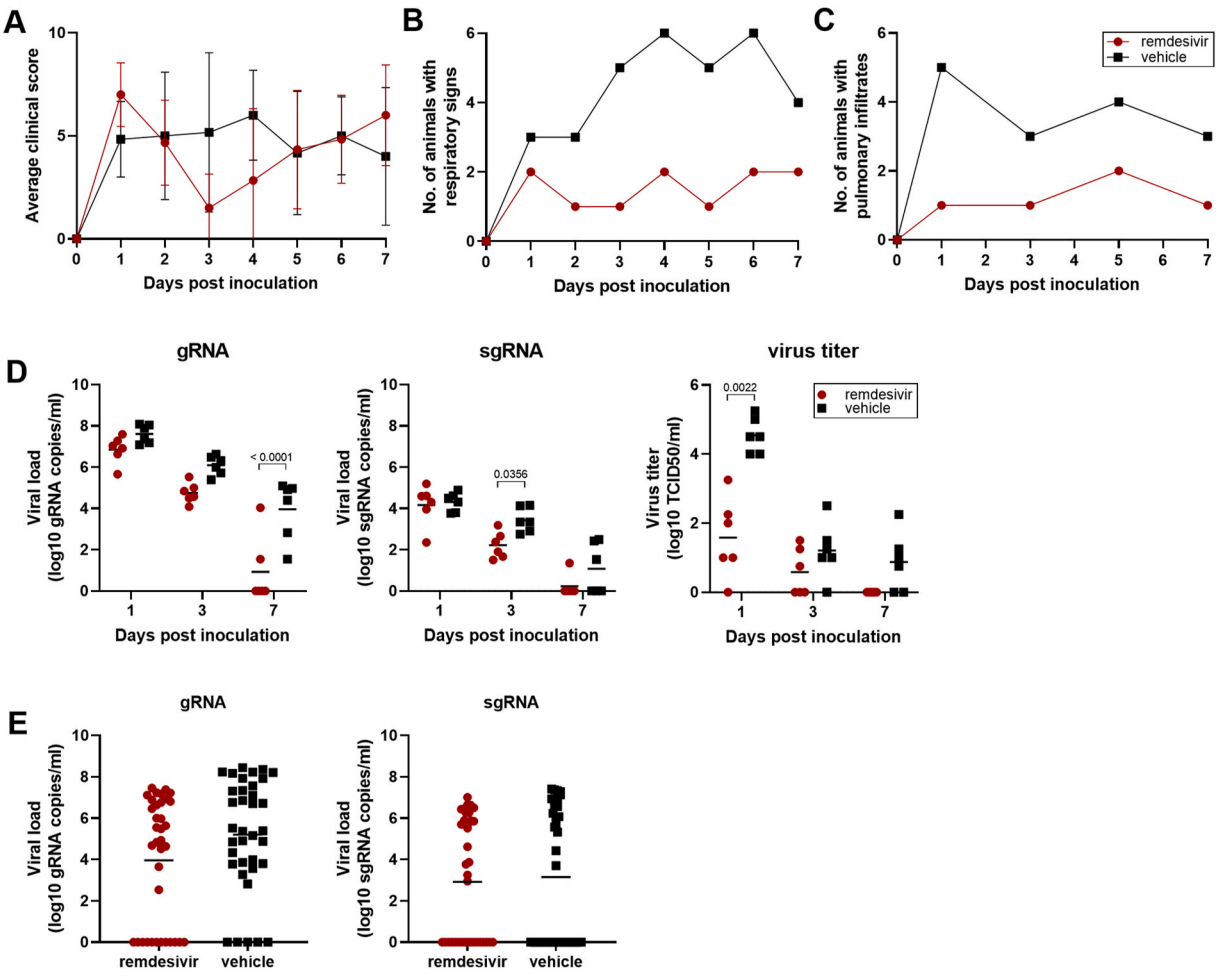

Fig. 1. Clinical disease and replication in the lower respiratory tract of rhesus macaques inoculated with SARS-CoV-2 and treated with subcutaneous remdesivir. Two groups of six rhesus macaques were inoculated with SARS-CoV-2 strain nCoV-WA1-2020. Twelve hours post inoculation, one group was administered 10 mg/kg subcutaneous remdesivir and the other group was treated with an equal volume of vehicle solution (NMP; 0.2 ml/kg). Treatment was continued 12 h after the first treatment, and every 24 h thereafter with a dose of 5 mg/kg remdesivir or equal volume of vehicle solution (0.1 ml/kg). A. Daily clinical scores were obtained using a standardized scoring sheet by a person blinded to the group assignment of the animals. B. Proportion of animals in each group that displayed respiratory signs during daily clinical scoring. C. Ventro-dorsal and lateral radiographs were scored for the presence of pulmonary infiltrates by a clinical veterinarian blinded to the group assignment of the animals according to a standard scoring system. The number of animals with pulmonary infiltrates over time is shown. D. On 1, 3, and 7 dpi, bronchoalveolar lavages were performed and tested for the presence of gRNA (left panel), sgRNA (middle panel) and infectious virus (right panel). E. On 7 dpi, animals were euthanized and tissue samples were collected from all lung lobes and tested for the presence of gRNA (left panel) and sgRNA (right panel). Data in panels A and D were analyzed using a 2-way ANOVA, and data in panel E using a Mann Whitney test; only p-values <0.05 are indicated in the graphs.

lower for remdesivir and greater for the alanine metabolite, indicative of a slower release of remdesivir from the injection site. Importantly, the active triphosphate (TP) metabolite levels in lungs at 24 h following either route of administration were not substantially different (Table S1). Therefore, we tested the efficacy of the subcutaneous formulation of remdesivir in the rhesus macaque model of SARS-CoV-2 infection (Munster et al., 2020).

Animal efficacy experiments were approved by the Institutional Animal Care and Use Committee of Rocky Mountain Laboratories, NIH and carried out in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility, according to the institution's guidelines for animal use, following the guidelines and basic principles in the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, United States Department of Agriculture and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. The Institutional Biosafety Committee (IBC) approved work with infectious SARS-CoV-2 strains under BSL3 conditions. Sample inactivation was performed according to IBC-approved standard operating procedures for removal of specimens from high containment.

Twelve Indian-origin rhesus macaques (11 females and 1 male; 4–7 years old) were randomly assigned to two groups of 6 animals. All animals were inoculated with a total dose of 2.6×10^6 TCID50 of SARS-CoV-2 strain nCoV-WA1-2020 (Vero E6 passage 4; no SNPs were detected in the inoculum at >5% by NGS) via intranasal (1 ml), oral (1 ml), ocular (0.5 ml) and intratracheal (4 ml) routes, as was done in our previous study assessing the efficacy of intravenous remdesivir administration (Williamson et al., 2020). At 12 h post inoculation (hpi), one group received a 10 mg/kg remdesivir loading dose via subcutaneous injection divided over two sites. Remdesivir solution was provided by Gilead Sciences as 50 mg/ml remdesivir in parenteral grade N-methyl-2-pyrrolidone (NMP). The second group of animals received an equivalent volume of NMP vehicle alone. At 24 hpi and every 24 h thereafter through 6 days post inoculation (dpi), treatment was continued with 5 mg/kg remdesivir or the equivalent volume of vehicle via a single subcutaneous injection. On 7 dpi, all animals were euthanized and skin was collected from the site of the last subcutaneous treatment. Injection site lesions were observed after subcutaneous injections with remdesivir and vehicle. Injection site lesions were generally more severe in remdesivir-treated animals; 5 out of 6

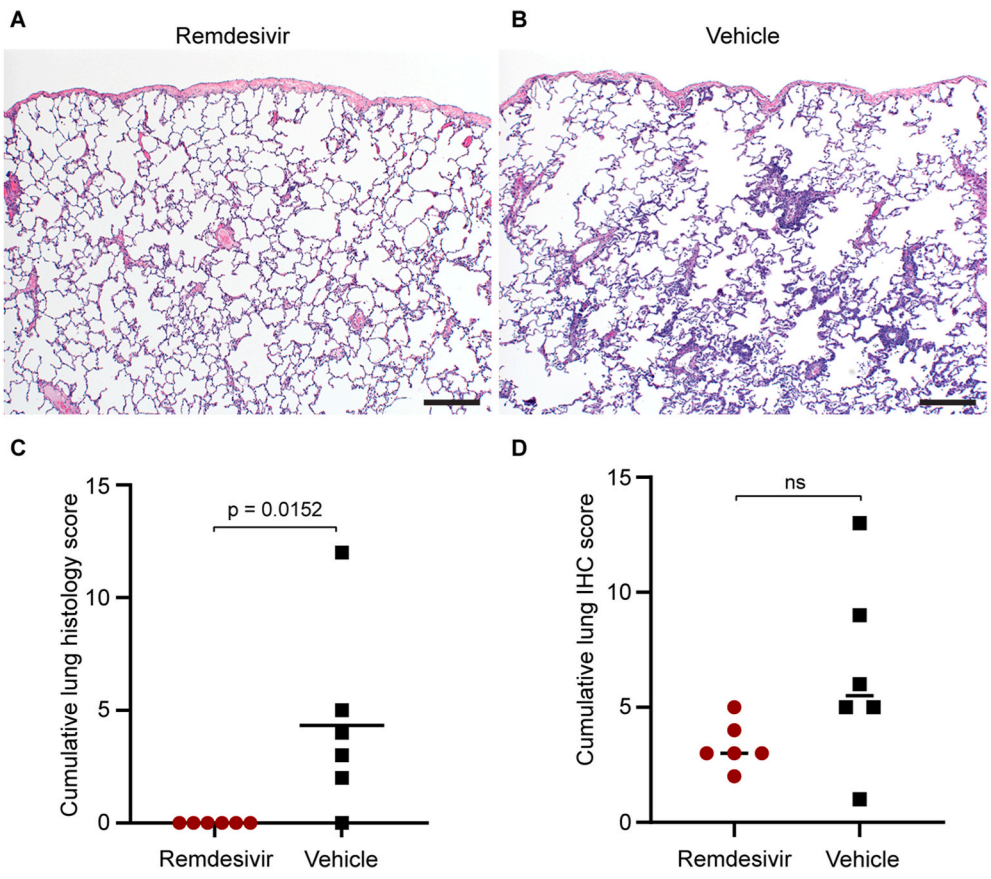

Fig. 2. Histological changes in the lungs of rhesus macaques inoculated with SARS-CoV-2 and treated with subcutaneous remdesivir. Two groups of six rhesus macaques were inoculated with SARS-CoV-2 strain nCoV-WA1-2020. Twelve hours post inoculation, one group was administered 10 mg/kg subcutaneous remdesivir and the other group was treated with an equal volume of vehicle solution (NMP; 0.2 ml/kg). Treatment was continued 12 h after the first treatment, and every 24 h thereafter with a dose of 5 mg/kg remdesivir or equal volume of vehicle solution (0.1 ml/kg). On 7 dpi, all animals were euthanized and lung tissue was collected for analysis by a board-certified veterinary pathologist blinded to the group assignment of the animals. A. Lungs from remdesivir-treated rhesus macaques were essentially normal. B. Multifocal interstitial pneumonia with lymphocytic perivascular cuffing in one of the vehicle-treated controls. C. Histological lesion severity was scored per lung lobe according to a standardized scoring system evaluating the presence of interstitial pneumonia, type II pneumocyte hyperplasia, edema and fibrin, and perivascular lymphoid cuffing (score 0–5); these values were combined per lung lobe and graphed. D. Presence of viral antigen was scored per lung lobe according to a standardized scoring system (0–5); these values were combined per lung lobe and graphed. The size bar in panel A and B indicates 200 μm. Statistical analysis was performed on the data in panel C and D using a Mann Whitney test; p-value < 0.05 is indicated in the graph; ns: not significant.

remdesivir-treated animals had mild to marked neutrophilic panniculitis and myositis with occasional myocyte necrosis, while 3 out of 6 vehicle-treated animals had minimal to mild panniculitis and myositis (Fig. S2). Phase I studies in humans would have to be performed to assess the severity of injection site lesions and whether their severity precludes use of subcutaneous administration of remdesivir.

Remdesivir and remdesivir metabolite levels were measured in the SARS-CoV-2-infected animals by LC-MS in plasma collected on 1, 3, 5 dpi just prior to the next treatment administration, as well as at the end of the experiment on 7 dpi, representing the circulating minimum concentrations throughout the dosage regimen. Intact remdesivir (GS-5734), alanine metabolite (GS-704277), and nucleoside metabolite (GS-441524) were readily detectable at all timepoints post-administration at levels similar to previous plasma measurements following intravenous remdesivir administration (Fig. S3A) (Warren et al., 2016). Plasma levels displayed higher concentrations of the GS-5734 prodrug compared to previous serum measurements made after intravenous remdesivir administration. At necropsy on 7 dpi, remdesivir and its metabolites were measured in lung tissue samples. Consistent with previous measurements made in a biocontainment setting (Williamson et al., 2020), only GS-441524 was detectable in lung samples since the sample analysis methodology employed in the biocontainment settings enables the detection and quantification of the nucleoside metabolite GS-441524, but does not allow for the detection of the intact active TP metabolite. GS-441524 was detected in all lung sections from all remdesivir-treated animals but the measured levels were in the lower range of values previously observed following intravenous administration (Fig. S3B). These data indicate that remdesivir was distributed to the lungs upon subcutaneous administration but accumulated to levels equivalent to or lower than those reached after intravenous administration, consistent with the pharmacokinetic analysis in healthy uninfected animals (Fig. S1).

After inoculation with SARS-CoV-2, the animals were scored daily for the presence of clinical signs according to a standardized scoring sheet (Munster et al., 2020) by a person blinded to the group assignment of the animals. Upon inoculation with SARS-CoV-2, the animals developed only mild signs of disease and no obvious differences in overall clinical scores were detected between the two groups (Fig. 1A). However, throughout the study, only 2 of 6 animals in the remdesivir-treated group developed respiratory disease signs (i.e. tachypnea and dyspnea), whereas all animals in the vehicle-treated group displayed respiratory signs (Fig. 1B). This correlated well with the presence of pulmonary infiltrates detected on radiographs, with 5/6 vehicle-treated animals having pulmonary infiltrates versus 2 remdesivir-treated animals (Fig. 1C). Thus, although the overall clinical scores were mainly determined by a lack of appetite observed in all animals that may have been affected by daily anesthesia required for drug administration, the treatment with subcutaneous remdesivir resulted in a reduction in respiratory disease. To monitor virus replication in the respiratory tract, nose and throat swabs were collected daily after inoculation and analyzed for the presence of SARS-CoV-2 genomic RNA (gRNA), as well as subgenomic RNA (sgRNA) as an indicator of recent virus replication. As with intravenous remdesivir treatment (Williamson et al., 2020), virus shedding from the upper respiratory tract was not affected by remdesivir treatment (Fig. S4). Subcutaneous remdesivir treatment reduced virus replication in the lower respiratory tract; on 3 and 7 dpi, significantly lower levels of sgRNA and gRNA, respectively, were detected in bronchoalveolar lavage (BAL) fluid of remdesivir-treated animals than that of vehicle controls (Fig. 1D). Additionally, there was a >100-fold reduction in virus titer in BAL fluid collected 12 h after the

first treatment administration, as was previously observed with intravenous remdesivir treatment (Williamson et al., 2020). On 7 dpi, all animals were euthanized and lung tissue was collected for virological and histological analyses. Although levels of gRNA and sgRNA were lower in lung lobes of remdesivir-treated animals, this difference was not statistically significant.

Histopathologic changes of mild interstitial pneumonia were detected in 5 out of 6 vehicle-treated animals, but not in any of the animals receiving subcutaneous remdesivir (Fig. 2A–C). Viral antigen could be detected in all animals regardless of treatment group (Fig. 2D). Together, our data indicate a reduction of respiratory signs, virus replication in the lower respiratory tract and a reduction in damage to the lungs with early initiation of subcutaneous remdesivir treatment.

One caveat of our study is that a direct comparison to our previous study on the efficacy of intravenous administration (Williamson et al., 2020) is impossible due to differences in the origin of rhesus macaques (Chinese-origin versus Indian-origin) that results in a difference in clinical signs, pulmonary infiltrates and histologic lung lesions. However, subcutaneous administration of remdesivir effectively prevented the development of lower respiratory tract disease, as did intravenous administration.

Recent clinical trial data showed a lack of improvement with remdesivir treatment in hospitalized patients (Ader et al., 2021). Although this may mean remdesivir treatment has limited effect in hospitalized patients, remdesivir treatment in non-hospitalized patients resulted in a significant reduction in hospitalizations (Gottlieb et al., 2021). Remdesivir is a direct-acting antiviral, and benefits of treatment are thus more likely to occur early during the infection when virus replication peaks, rather than during severe disease that is largely immune-driven. SARS-CoV-2 neutralizing monoclonal antibody therapies have shown great benefit when used to prevent rather than treat severe COVID-19 (reviewed in (Hurt and Wheatley, 2021)). Likewise, the development of new remdesivir formulations that are easier to administer in outpatient settings may result in an increased benefit of treatment. Since remdesivir is a broad-acting antiviral with efficacy against paramyxoviruses and filoviruses besides coronaviruses (Lo et al., 2017), the identification of alternative administration routes for remdesivir could have a much broader impact than on COVID-19 patients alone.

Data availability

Data included in this manuscript have been deposited in Figshare: https://figshare.com/articles/dataset/Subcutaneous_remdesivir_administration_prevents_interstitial_pneumonia_in_rhesus_macaques_inoculated_with_SARS-CoV-2/16900057.

Declaration of competing interest

BNW, LP-P, BS, FF, MH, MS, EH, AO, JL, CMB, GS and EdW have no conflicts to declare, DSL, DB, RS, DPP, RLM and TC are employees of Gilead Sciences and own company stock.

Acknowledgements

We thank Tina Thomas, Dan Long, Rebecca Rosenke, Marissa Woods, Kathy Cordova, Carl Shaia and Vincent Munster for technical assistance; RMVB animal care staff for taking care of the animals, and Anita Mora for help with figure preparation. This study was supported by the Intramural Research Program of NIAID, NIH.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2022.105246.

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