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Late remdesivir treatment initiation partially protects African green monkeys from lethal Nipah virus infection

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Abstract

Remdesivir is a nucleotide prodrug with preclinical efficacy against lethal Nipah virus infection in African green monkeys when administered 1 day post inoculation (dpi) (Lo et al., 2019). Here, we determined whether remdesivir treatment was still effective when treatment administration initiation was delayed until 3 dpi. Three groups of six African green monkeys were inoculated with a lethal dose of Nipah virus, genotype Bangladesh. On 3 dpi, one group received a loading dose of 10mg/kg remdesivir followed by daily dosing with 5mg/kg for 11 days, one group received 10mg/kg on 12 consecutive days, and the remaining group received an equivalent volume of vehicle solution. Remdesivir treatment initiation on 3 dpi provided partial protection from severe Nipah virus disease that was dose dependent, with 67% of animals in the high dose group surviving the challenge. However, remdesivir treatment did not prevent clinical disease, and surviving animals showed histologic lesions in the brain. Thus, early administration seems critical for effective remdesivir treatment during Nipah virus infection.

Keywords

Nipah virus; antiviral; remdesivir; nonhuman primate; nucleotide analog

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Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E.d.W., B.N.W., F.F., K.G., M.K.L., A.O., J.L., G.S., C.F.S., and H.F. have no conflicts to declare. E.B., D.P.P. and T.C. are employees of Gilead Sciences and own company stock.

Remdesivir is a nucleotide prodrug with antiviral activity against a broad range of RNA viruses, including filoviruses, coronaviruses, and paramyxoviruses (Lo et al., 2017; Sheahan et al., 2017; Warren et al., 2016). After the emergence of SARS-CoV-2, remdesivir was the first antiviral to receive FDA Emergency Use Authorization, and later full approval, for the treatment of COVID-19 patients (Aleissa et al., 2020). Remdesivir treatment is currently recommended for use in non-hospitalized patients at risk of developing severe COVID-19 as well as in hospitalized COVID-19 patients (Health, 2021).

Nipah virus is a paramyxovirus that causes severe respiratory and neurological disease with a case-fatality rate around 70% (Ang et al., 2018). No vaccines or antivirals are currently approved to prevent or treat Nipah virus infection. We have previously shown that remdesivir is an effective treatment in African green monkeys infected with Nipah virus, genotype Bangladesh. Daily intravenous administration of 10mg/kg remdesivir on 1–12 days post inoculation (dpi) resulted in 100% survival until the end of the study on 92 dpi (Lo et al., 2019). However, disease progression in Nipah virus patients is often very rapid after diagnosis (Nikolay et al., 2019), and 1 dpi treatment initiation likely does not reflect a feasible treatment scenario in the majority of patients. Therefore, we decided to determine the efficacy of remdesivir treatment when administration was initiated later.

We further defined the efficacy of remdesivir against Nipah virus, genotype Bangladesh in African green monkeys with treatment initiation on 3 dpi. These experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Rocky Mountain Laboratories, NIH and carried out in an 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. Sample inactivation was performed according to IBC-approved standard operating procedures for removal of specimens from high containment.

Three groups of six adult African green monkeys (3.2–5.7kg; 9 males and 9 females divided evenly over the experimental groups) were inoculated with 10^5 50% tissue culture infectious dose (TCID50) intranasally (0.5ml per nostril) and 10^5 TCID50 intratracheally (4ml) as previously described (Lo et al., 2019; Mire et al., 2016). At 3 dpi, remdesivir treatment was initiated. All treatments were delivered by slow (5 minute) infusion). The first group (hereafter 'RDV 5mg/kg') of animals received a 10 mg/kg loading dose of remdesivir in vehicle solution (5mg/ml in 12% sulfobutylether-β-cyclodextrin in water and hydrochloric acid, pH3.5) as a slow intravenous bolus injection on 3dpi, followed by 5mg/kg remdesivir in vehicle solution on 11 consecutive days (4–14dpi). The second group (hereafter 'RDV 10mg/kg') received 10 mg/kg of remdesivir in vehicle solution on 3–14dpi; while the third group (hereafter 'vehicle') received either (n=3) the same volume of vehicle solution as animals in the first (2 ml/kg on 3dpi followed by 1ml/kg 4–14 dpi) or (n=3) the second (2ml/kg 3–14dpi) group. The two doses of remdesivir were tested to match the dose successfully used to test the efficacy of remdesivir in a rhesus macaque model of SARS-CoV-2 infection (Williamson et al., 2020) that more closely reflects the recommended

dosing in COVID-19 patients (RDV 5mg/kg) and the dose used in our previous Nipah virus study where treatment was initiated on 1 dpi (RDV 10mg/kg) (Lo et al., 2019).

After inoculation with Nipah virus, animals were monitored daily for signs of disease and assigned a clinical score based on an IACUC-approved standardized scoring sheet by a person blinded to the group assignment of the animals (Munster et al., 2020); a clinical score ≥35 requires euthanasia. The six vehicle-treated control animals reached endpoint criteria and were euthanized between 6–8 dpi (Fig. 1A). Four out of six animals in the RDV 5mg/kg group were euthanized between 7–9 dpi, while only two animals in the RDV 10mg/kg group reached endpoint criteria on 9 and 11 dpi (Fig. 1A). All animals in the study showed obvious disease signs starting 3–5 dpi (Fig. 1B), including loss of appetite, lethargy, ruffled fur, and hunched posture; tachypnea and dyspnea were exclusive to the vehicle group and started on 4 dpi. Whereas the six vehicle-treated control animals all developed severe respiratory disease, with overt neurological signs in only one animal, the remdesivir-treated animals displayed less severe respiratory signs but a higher prevalence of neurological signs (Fig. S1A) including tremors, unusual behavior, uncoordinated movements, balance problems, and partial paralysis. This is in line with previous observations in animal models, where animals that do not succumb to respiratory disease later develop neurological disease signs (Liu et al., 2019; Munster et al., 2012; Rockx et al., 2011). Anisocoria, a possible neurological sign, was observed in two animals in the RDV 10mg/kg group: in one animal on 9 dpi when it was examined prior to euthanasia, and on 35 and 42 dpi in an animal that was otherwise healthy.

Viral loads in fluid and tissue samples were determined by qRT-PCR as described previously (Lo et al., 2019). All animals became viremic after inoculation, regardless of treatment status (Fig. S1B). However, the viral load in blood was statistically significantly lower in animals treated with remdesivir that survived the Nipah virus challenge versus those that reached endpoint criteria, even before treatment initiation (Fig. 1C).

Tissue samples and cerebrospinal fluid (CSF) were collected at necropsy following euthanasia from all animals. Viral RNA was detected in the CSF of two out of six control animals, as well as in all animals treated with remdesivir that reached endpoint criteria before the study end, regardless of treatment dose (Fig. 1D). This likely reflects the fact that control animals developed severe respiratory disease requiring euthanasia, while remdesivir-treated animals developed neurological signs. Viral RNA was not detected in the CSF collected at necropsy from remdesivir-treated animals that survived until the study endpoint on 42 dpi (Fig. 1D). The viral loads were highest in the lungs of vehicle-treated animals and animals in the RDV 5mg/kg group that reached endpoint criteria before the study endpoint (Fig. 1E). Tissue samples collected from the CNS at euthanasia also contained high viral loads in all animals that reached endpoint criteria before the study end, regardless of treatment received (Fig. 1E). Interestingly, viral RNA was also detected in the CNS of all animals that survived until the study end, especially in the brainstem.

At necropsy, tissue samples were collected for histologic analysis by a board-certified veterinary pathologist blinded to the group assignment of the animals. Histologically, the lungs of in the RDV 5mg/kg group that reached endpoint criteria before the study end and of

all animals in the vehicle-treated group were characterized by bronchointerstitial pneumonia with abundant alveolar and perivascular edema; alveolar septa were often expanded by fibrin, lymphocytes, plasma cells, and fewer neutrophils (Fig. 2). In the CNS of all animals in the RDV 5mg/kg group that reached endpoint criteria before the study end and in one of the vehicle-treated animals there were rare mild regions of nonsuppurative perivascular cuffing (Fig. 2). In one of two of animals in the RDV 5mg/kg group that survived to the end of the experiment, and five of six animals in the RDV 10mg/kg group, there were varying degrees of nonsuppurative perivascular cuffing, meningoencephalitis, gliosis, and rarely malacia in the CNS. There were no significant findings in the lungs of these animals (Fig. 2).

To evaluate whether remdesivir treatment failure in the six animals that were euthanized before study endpoint may have been due to resistance mutations, we sequenced Nipah virus genomes from the right lower lung lobe and cerebellum of all animals in the vehicle control group and all animals treated with remdesivir that succumbed to infection. Viral loads in tissues of animals treated with remdesivir that survived Nipah virus challenge were too low to generate sequences. Samples were processed and analyzed as described in (Munster et al., 2021) with the following changes: the Illumina Stranded Total RNA Ligation with Ribo-Zero Plus (Illumina) with 2ug RNA input and 14 PCR amplification cycles was used for library preparation; myBaits® Custom Virus panel NiV/CedV/HeV and following the manufacturer's manual, version 5.02 with 9 – 14 cycles of post-capture PCR amplification was used for RNA capture in cerebellum samples due to low viral loads in these samples; MiSeq v2–300cycle (2 × 150) was used on the Illumina MiSeq instrument. Although several minor variants were found in the sequences from vehicle control animals, as well as in remdesivir-treated animals (Table S1), none of these changes were in the NiV polymerase gene L, where resistance-associated changes would be expected.

Late remdesivir treatment initiation (3 dpi) still provided partial protection from severe Nipah virus disease that was dose dependent. Treatment success was also associated with lower viral loads in blood at time of treatment initiation, since all remdesivir-treated animals that did not survive Nipah virus infection had detectable viremia, where five out six surviving animals did not. This is similar to remdesivir treatment in Ebola virus disease patients, where lower viral loads at time of treatment initiation resulted in increased survival (Mulangu et al., 2019). Although the higher, 10mg/kg remdesivir dose resulted in increased survival and reduced respiratory disease, it did not completely prevent histologic lesions in the CNS and associated neurologic signs of disease during the acute stage of infection. While surviving animals in the 10mg/kg treatment group had histologic lesions in the CNS, no overt neurological signs were observed in these animals at study end, except anisocoria of unknown cause in one animal. Thus, it is not clear whether lesions in surviving animals would have resolved or progressed to overt disease or a potential relapse at a later timepoint. The fact that viral loads in tissues of the CNS were higher in animals euthanized early in the study than in animals who survived until the end of the study may indicate that Nipah virus infection in the CNS was resolving, or at least not progressing. Overt neurological disease is rarely observed in African green monkeys inoculated with Nipah virus, since the respiratory disease is rapidly fatal. As such, this study provides tissues to study the pathology of acute Nipah virus neurological disease in the future.

One limitation of this study is the inability to monitor surviving animals for a longer period after Nipah virus inoculation for potential relapses, due to the animal welfare and logistical challenges of performing these studies in BSL4. Moreover, additional data on the efficacy of remdesivir treatment on 2 dpi would have resulted in a more precise understanding of the remdesivir treatment window in Nipah virus infection. However, since human data that would allow bridging of animal model data to human disease progress are lacking, these additional treatment timepoint would still not give an exact idea of when to administer remdesivir in patients.

Our findings indicate that remdesivir treatment as it is currently used in COVID-19 patients, would be most effective in Nipah virus patients who are still in the early disease stage. To improve treatment effect, administering a higher dose of remdesivir could be considered. There is precedent to treat encephalitis with a higher dose of remdesivir than the currently used dosage in COVID-19. A case of meningoencephalitis from an Ebola virus relapse was treated daily for 14 days with 225mg remdesivir (Jacobs et al., 2016); this is higher than the currently recommended human dosing of 200mg remdesivir loading dose followed by 100mg daily. However, other than this single patient, there are no safety data to support the use of this treatment regimen. Alternatively, remdesivir treatment could be combined with other treatment options such as m102.4 monoclonal antibody treatment, which has shown efficacy in African green monkeys infected with Nipah virus, genotype Bangladesh, and was successfully tested in a phase 1 human clinical trial (Mire et al., 2016; Playford et al., 2020).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data included in this manuscript have been deposited in Figshare: https://doi.org/10.6084/m9.figshare.22325833

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Highlights

- Remdesivir is a nucleotide analog prodrug with broad-spectrum antiviral activity shown to be effective against Nipah virus.
- The efficacy of remdesivir treatment initiation on 3 days post inoculation was tested in the African green monkey model.
- Late remdesivir treatment resulted in partial protection and efficacy was dose dependent.
- Remdesivir treatment shifted the Nipah virus disease manifestations towards neurological disease

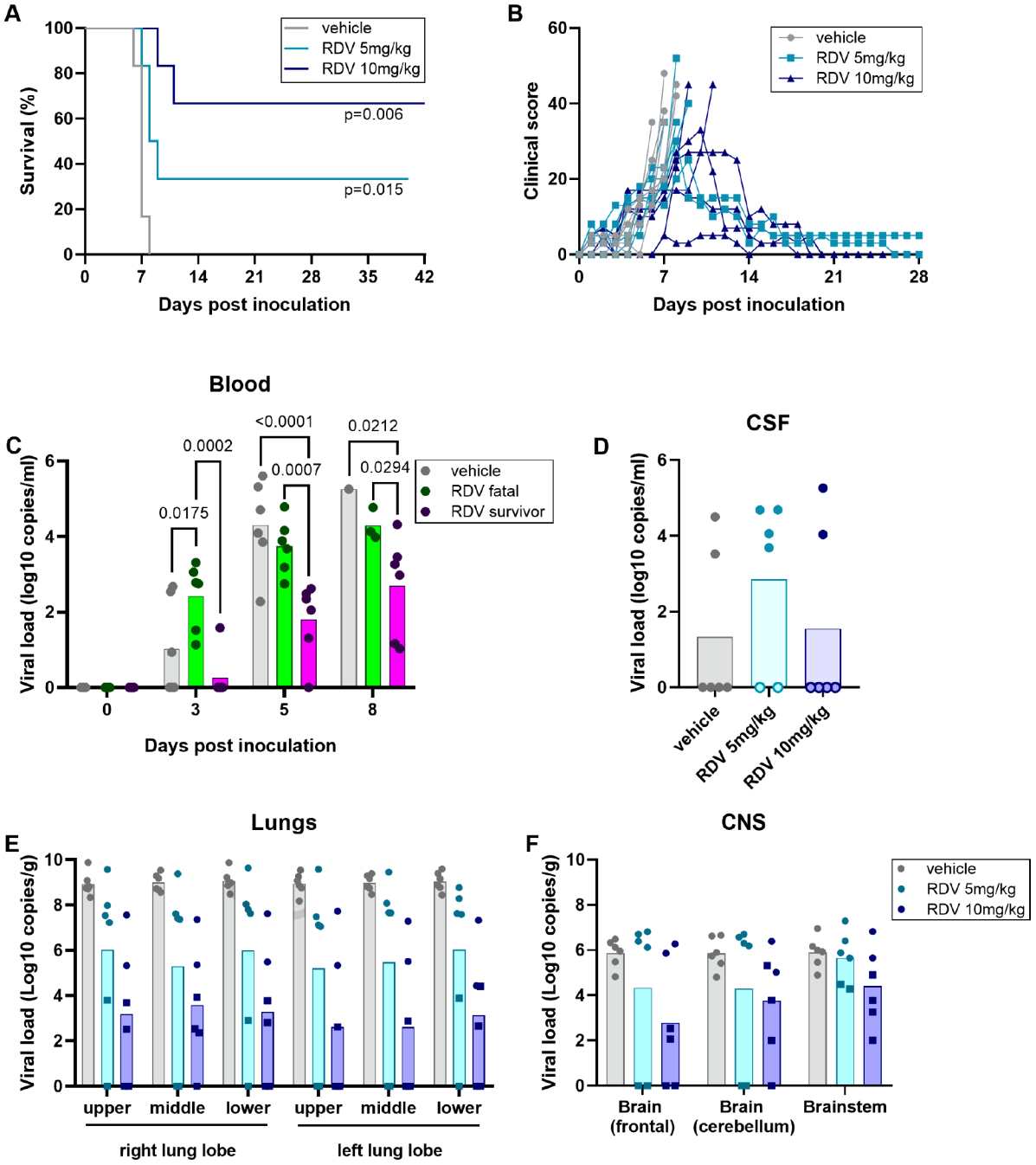

Figure 1. Effect of remdesivir treatment on Nipah virus infection in African green monkeys.

Three groups of six African green monkeys were inoculated with Nipah virus, genotype Bangladesh; remdesivir treatment was initiated 3 days later. One group received a loading dose of 10mg/kg remdesivir followed by daily dosing with 5mg/kg for 11 days (RDV 5mg/kg), one group received 10mg/kg for 12 consecutive days (RDV 10mg/kg), and the remaining group received an equivalent volume of vehicle solution (vehicle). A. Survival in animals inoculated with a lethal dose of Nipah virus, genotype Bangladesh and treated with remdesivir or vehicle. Statistical significance compared to the vehicle group was calculated

in a log-rank (Mantel-Cox) test. B. Daily clinical scores were obtained by a person blinded to the group assignment of the animals using a standardized scoring sheet. C. Whole blood was collected from all animals in study on 0, 3, 5, and 8 dpi and analyzed for the presence of viral RNA. Data from animals treated with remdesivir are grouped by outcome (endpoint criteria reached before study endpoint vs. survival until the end of the experiment). In each run, counted RNA standards were included to calculate the copy number in the samples. Statistical analysis was performed using a 2-way ANOVA with Tukey's multiple comparisons tests; p-values <0.05 were considered significant and are indicated. D. At euthanasia, cerebro-spinal fluid (CSF) was collected from all animals and analyzed for the presence of viral RNA. Open symbols indicate animals that survived until study end; closed symbols indicate animals that reached endpoint criteria and were euthanized before study endpoint. E. Lung tissue samples were collected from each lung lobe and (F) from three regions of the central nervous system and analyzed for the presence of viral RNA. Squares indicate animals that survived until study end; circles indicate animals that reached endpoint criteria and were euthanized before study endpoint.

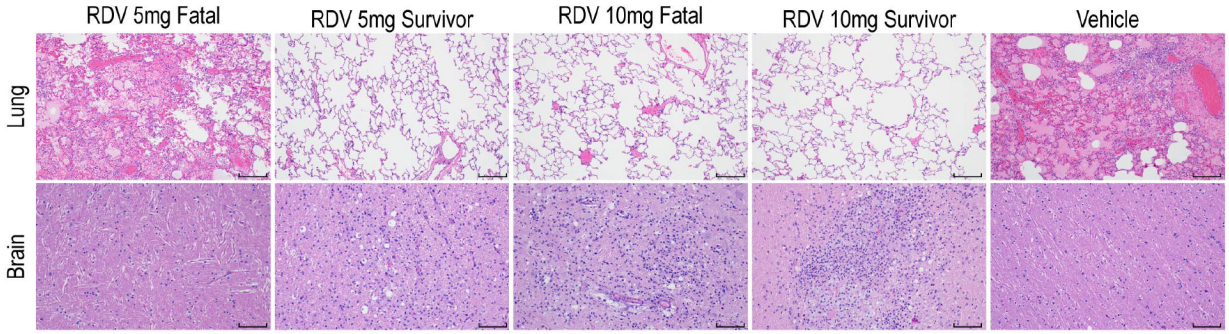

Figure 2. Histopathologic findings in the lungs and brains of remdesivir-treated animals.

In the animals in the RDV 5mg/kg group that reached endpoint criteria before the study end (n=4) and in all animals in the vehicle-treated group (n=6), the lungs were characterized by abundant alveolar and perivascular edema, fibrin accumulation, and marked bronchointerstitial pneumonia. In the CNS of all animals that in the RDV 5mg/kg group that reached endpoint criteria before the study end (n=2) and in the CNS of one of the vehicle-treated animals there were rare mild regions of perivascular cuffing. In one of the two of animals in the RDV 5mg/kg group that survived to the end of the experiment, and five of six animals in the RDV 10mg/kg group, there were varying degrees of nonsuppurative perivascular cuffing and/or meningoencephalitis in the CNS and no significant findings in the lungs. Lung magnification 100x, scale bar 200μm. Brain magnification 200x, scale bar 100μm. The number of animals with and without histologic lesions in lungs and brains in each group is indicated in Fig. S1C.