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Dexamethasone treatment does not alter mortality but reduces pulmonary pathology in Nipah virus-infected Syrian hamsters

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Abstract

Nipah virus (NiV) is an emerging zoonotic pathogen that causes severe respiratory and neurologic disease, and there are currently no licensed vaccines or approved treatments. The acute respiratory disease caused by NiV is associated with severe inflammation, similar to severe COVID-19. Dexamethasone is an affordable and widely available synthetic glucocorticoid, that improved outcomes when administered to patients with severe COVID-19. To determine whether a similar beneficial effect could be achieved during NiV infection, we tested the effect of an anti-inflammatory or immunosuppressive dose of dexamethasone on NiV in the Syrian hamster model. We found that dexamethasone treatment produced the expected hematologic changes in uninfected animals in a dose-dependent manner. In NiV-infected animals, the anti-inflammatory dose of dexamethasone reduced pulmonary pathology, while the immunosuppressive dose had no effect. The anti-inflammatory dose did not increase virus replication in tissues or virus shedding from the respiratory tract, indicating the anti-inflammatory dose of dexamethasone does not result in increased virus replication. Despite reduced lung pathology, dexamethasone treatment did not increase survival after NiV challenge. When dexamethasone treatment was combined with the antiviral remdesivir, dexamethasone negated the increased survival observed in hamsters treated with remdesivir alone. Our study provides critical information on the effect of dexamethasone administration on the outcome of NiV infection and cautions against the use of dexamethasone in combination with other antivirals like remdesivir without preclinical validation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

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Keywords

Nipah virus; Therapeutics; Animal model; Treatment efficacy; Immunopathogenesis; Immunomodulatory treatment

1. Introduction

Nipah virus (NiV) is an emerging zoonotic pathogen that causes severe respiratory and neurologic disease (Ang et al., 2018; Hossain et al., 2008), with an average mortality of ~70 % (Bhowmik et al., 2025; Ching et al., 2015; Chua et al., 1999). Currently, there are no licensed vaccines to prevent NiV infection, and no approved treatments, therefore patient treatment is generally supportive in nature. Combined with its ability to transmit between people, effective treatments are critically needed.

NiV disease patients present with a spectrum of clinical signs and symptoms, primarily respiratory distress and neurologic disease. Neurologic signs include altered mental status, seizures, and coma. In the brain, NiV causes a vasculitis, neuronophagia, and non-suppurative meningoencephalitis (Chua et al., 1999). NiV causes a severe inflammatory disease in the lungs, characterized by vasculitis, increased vascular permeability, fibrin exudation, infiltrating leukocytes, and edema (Wong et al., 2002). During the COVID-19 pandemic, a proportion of infected individuals developed severe acute respiratory disease, which necessitated hospitalization and respiratory support (Bi et al., 2020; Chen et al., 2020). Dexamethasone, a synthetic glucocorticoid, was shown to reduce the incidence of death from 41.4 % to 29.3 % in COVID-19 patients receiving mechanical ventilation (Group et al., 2021). Dexamethasone has numerous effects on tissues and leukocytes and is a potent anti-inflammatory drug (Strehl et al., 2019). Early during infection, SARS-CoV-2 and NiV cause similar acute lung injuries, characterized by pulmonary edema, fibrin exudation and infiltrating leukocytes (Borczuk, 2021). Thus, we reasoned that dexamethasone may mitigate the immunopathogenesis associated with NiV-induced respiratory disease. Dexamethasone is affordable and widely available, adding to its appeal as a potential treatment for NiV disease.

Here, we tested the efficacy of anti-inflammatory and immunosuppressive doses of dexamethasone to reduce Nipah virus disease severity in Syrian hamsters. Syrian hamsters develop severe respiratory and central nervous system (CNS) disease after inoculation with NiV (DeBuysscher et al., 2013). Additionally, Syrian hamsters were used to demonstrate the beneficial effects of dexamethasone treatment in SARS-CoV-2 infection (Merle-Nguyen et al., 2024), alone or as part of combined treatment with an antiviral drug (Ye et al., 2021) or monoclonal antibodies (Wyler et al., 2022). Similarly, we examined the effect of dexamethasone in Syrian hamsters infected with NiV, both as a single treatment and combined with the antiviral remdesivir, a nucleotide analog shown to improve survival of Nipah virus disease in African green monkeys (de Wit et al., 2023b; Lo et al., 2019). We show that anti-inflammatory doses of dexamethasone reduce pulmonary pathology in Syrian hamsters, but do not improve survival, even in combination with remdesivir treatment.

2. Materials and methods

2.1. Ethics statement

Approval of animal experiments was obtained from the Institutional Animal Care and Use Committee of the Rocky Mountain Laboratories, National Institutes of Health. Experiments were 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 NIH Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, U.S. Department of Agriculture, and the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Work with infectious Nipah virus under BSL4 conditions was approved by the Institutional Biosafety Committee (IBC). Inactivation and removal of samples from high containment was performed per IBC-approved standard operating procedures.

2.2. Study design

Syrian hamsters were chosen for this study since they recapitulate human Nipah virus disease reasonably well, have been used to show effect of dexamethasone treatment previously, and because the use of hamsters enables the use of larger groups sizes necessary to reach statistical power. To assess whether dexamethasone was effective in Syrian hamsters, we first tested the effect of a dose of dexamethasone considered anti-inflammatory (0.2 mg/kg) or immunosuppressive (2 mg/kg) on circulating leukocytes (Carpenter and Harms, 2022). Two groups of six hamsters were treated with 0.2 mg/kg dexamethasone or 2 mg/kg dexamethasone for 5 days; one group of two control hamsters received phosphate buffered saline (PBS). Animals were euthanized 2 days after the last treatment, blood collected and hematology performed.

To determine the effect of dexamethasone treatment on the outcome of Nipah virus infection, we next tested both treatment doses in Syrian hamsters inoculated with NiV-Malaysia (NiV-M). Because data from COVID-19 patients showed that treatment initiation time can drastically impact treatment effect (Bi et al., 2020; Liu et al., 2024), we tested two timepoints of treatment initiation: 2 and 4 days post inoculation (dpi). Five groups of six Syrian hamsters were inoculated intranasally with NiV-M, and treated as follows: 0.2 mg/kg dexamethasone on 2–6 dpi, 0.2 mg/kg dexamethasone on 4–8 dpi, 2 mg/kg dexamethasone on 2–6 dpi, 2 mg/kg dexamethasone on 4–8 dpi, or a volume of PBS equivalent to the 2 mg/kg dose on 4–8 dpi.

Since we observed reduced lung pathology and a trend towards improved survival with 0.2 mg/kg dexamethasone treatment on 4–8 dpi, we repeated this group to increase the statistical power of our experiment. However, since we also observed that lethality mostly occurred after dexamethasone treatment was stopped, we also increased the treatment window. Two groups of 10 Syrian hamsters were inoculated with NiV-M; one group was treated with 0.2 mg/kg dexamethasone on 4–13 dpi, while one group of control animals received an equivalent volume of PBS. An additional four Syrian hamsters/group were included and euthanized on 6 dpi to compare viral loads and histopathologic changes in tissues.

Remdesivir has been used off-label to treat Nipah virus patients in India (As et al., 2024). To define a dose of remdesivir where ~50 % of Syrian hamsters survived NiV-M infection, three groups of 10 animals were inoculated with NiV-M. On 1–7 dpi, one group received 20 mg/kg remdesivir, one 25 mg/kg remdesivir, and one a volume of vehicle solution equivalent to a 25 mg/kg dose. An additional four Syrian hamsters/group were included in the remdesivir-treated groups and euthanized on 6 dpi to compare viral loads and histopathologic changes in tissues. The six dpi timepoint was chosen since it was the latest timepoint before large numbers of animals in the previous experiment started to reach endpoint criteria.

To assess the efficacy of combined dexamethasone and remdesivir treatment, 4 groups of 10 Syrian hamsters were inoculated with NiV-M and treated as follows: 0.2 mg/kg dexamethasone on 4–13 dpi; 25 mg/kg remdesivir on 1–7 dpi; 0.2 mg/kg dexamethasone on 4–13 dpi and 25 mg/kg remdesivir on 1–7 dpi; an equivalent volume of PBS on 4–13 dpi and an equivalent volume of vehicle on 1–7 dpi. An additional four Syrian hamsters/group were included and euthanized on 6 dpi to compare viral loads and histopathologic changes in tissues.

2.3. Animal experiments

Equal numbers of male and female Syrian hamsters (Envigo), at least six-weeks-old, were randomly assigned to experimental groups. Under isoflurane anesthesia, hamsters were inoculated intranasally with 10^6 $TCID_{50}$ of NiV-M in a volume of 50 μl diluted in sterile Dulbecco's Modified Eagle Medium (DMEM). The inoculum was divided over both nares by pipetting. For experiments involving NiV inoculation, nasal and oropharyngeal swabs were collected every other day in 1 ml DMEM. At the time of euthanasia (6 dpi, when animals reached endpoint criteria, or 28 dpi), blood and tissues were collected. A mid-line sagittal section of brain was formalin fixed, allowing for examination of the cerebrum, cerebellum, midbrain and brainstem. The lungs were insufflated with formalin and fixed en bloc, to allow for examination of all lung lobes.

Animals receiving dexamethasone treatment were injected subcutaneously once daily with dexamethasone sodium phosphate 4 mg/ml (VetOne) diluted in sterile PBS and administered in a volume not exceeding 0.5 ml. Control groups received the same volume of PBS. Animals receiving remdesivir were injected once daily intraperitoneally (Ye et al., 2021) with research grade >99 % pure remdesivir (MedChemExpress) dissolved in aseptic Captisol (Ligand Pharmaceuticals) with 2.5 % DMSO, sterile filtered using a 0.22 μm syringe filter (Millipore), in a volume not exceeding 1 mL. Control groups received the same volume of Captisol.

The experimental endpoint criteria requiring euthanasia for this study were defined as: a fixed timepoint (6dpi or 28dpi) or if the animals demonstrated weight loss >20 %, or respiratory distress, or paralysis, torticollis, seizures. Animals were euthanized under deep anesthesia via bilateral thoracotomy and/or cervical dislocation using a mechanical device.

2.4. Virus and cells

Nipah virus strain Malaysia/199901924 (GenBank AF212302; cell culture passage 2 obtained from the Special Pathogens Branch of the Centers for Disease Control and Prevention) was propagated once in Vero E6 cells in DMEM supplemented with 2 % fetal bovine serum (FBS), 1 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. Next generation sequencing confirmed the sequence of the virus stock was identical to that in GenBank and no contaminants were present. VeroE6 cells were cultured in DMEM, 10 % FBS, 1 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. Cells were tested monthly for the presence of mycoplasma and remained negative.

2.5. Hematology

EDTA blood was collected and run on a ProCyte DX (IDEXX Laboratories) to obtain hematology data.

2.6. RNA extraction and qRT-PCR

RNA was extracted from swabs using the QIAamp Viral RNA Mini Kit (Qiagen) and from 30 mg tissues using the RNeasy Mini Kit (Qiagen), according to manufacturer's instructions and as described previously (de Wit et al., 2023a). Nipah virus nucleoprotein gene (N) RNA was quantitated using one-step qRT-PCR (de Wit et al., 2023a) with 2 μl of RNA input conducted using the QuantiNova Probe RT-PCR kit (Qiagen) according to the manufacturer's instructions and run on a QuantStudio 6 instrument (Thermo Fisher Scientific). Serial dilutions of RNA standards with known copy numbers were run in parallel in each run to calculate RNA copy numbers in the samples.

2.7. Histology and immunohistochemistry

Tissues were fixed in 10 % neutral-buffered formalin ×2 changes, for a minimum of 7 days. Embedded tissues were sectioned at 5 μm and dried overnight at 42 °C prior to staining. Sections were stained with hematoxylin and eosin stain (H&E).

Nipah virus antigen was detected using a rabbit polyclonal anti-Nipah virus nucleoprotein antibody (GeneTex) at a 1:1000 dilution. ImmPRESS VR horse anti-rabbit IgG polymer (Vector Laboratories) was used as a secondary antibody. For negative controls, replicate sections from each block were deparaffinized and stained in parallel following an identical protocol, with the primary antibody replaced by rabbit IgG (Vector Laboratories) at a dilution of 1:2500.

A near-midline sagittal section was examined of the brain, allowing for visualization of the cerebrum, cerebellum, pons, midbrain, and medulla. Histologic lesions from brain sections stained with H&E were categorized as described previously (Singh et al., 2024) into lymphoplasmacytic meningitis; lymphoplasmacytic encephalitis with gliosis; malacia; vasculitis and fibrin thrombi; and hemorrhage. Each category was scored on the scale of 0 = none,1 = rare, 2 = mild, 3 = moderate and 4 = severe; scores per category were added up to reach a cumulative histology score.

After removing a small portion of the right lung lobes for virology testing, the lungs were insufflated with 10 % neutral-buffered formalin via the trachea, and placed in the cassette whole, allowing for examination of all lung lobes, trachea, and mediastinal structures, such as lymph nodes. Histologic lesions from lung sections stained with H&E were categorized as described previously (Singh et al., 2024) into lymphoid aggregates; lymphoplasmacytic interstitial pneumonia; presence of syncytial cells; interstitial and intra-alveolar macrophages; type II pneumocyte hyperplasia; perivascular and alveolar edema; vasculitis, and fibrin thrombi. Each category was scored on the scale of 0 = none, 1 = rare, 2 = mild, 3 = moderate and 4 = severe; scores per category were added up to reach a cumulative histology score. Immunohistochemistry scoring was as follows 0 = no positive cells; 1 = rare/minimal positive cells; 2 = mild/few positive cells; 3 = moderate numbers of positive cells; 4 = abundant positive cells; 5 = severe/marked positive cells; one immunohistochemistry score was assigned per tissue. All scoring was performed by a board-certified veterinary pathologist.

2.8. Quantification of hamster innate immune gene expression

RNA was extracted from tissues as described above. cDNA was generated using the SuperScript IV VILO kit with EZDNase (ThermoFisher Scientific) according to the manufacturer's instructions. qPCR to detect expression of IFN-γ, IL-1β, IL-6, TNFα, and RIG-I was performed using the TaqMan Fast Advanced Master Mix (ThermoFisher) on a QuantStudio 6 instrument (ThermoFisher Scientific). β-2-M and RPL-18 were used as endogenous controls for relative quantification analysis using the $2^{-\Delta Ct}$ method; data were normalized to the expression detected in the control group treated with PBS and vehicle. Primer/probe sequences were taken from (Zivcec et al., 2011).

2.9. ELISA

IgG antibody responses were measured in an ELISA using recombinantly expressed NiV-M glycoprotein G, as described previously (de Wit et al., 2023a; Singh et al., 2024) with small modifications. Briefly, Nunc Maxisorp plates (ThermoFisher) were coated with recombinant NiV G (50 ng in 100 µl per well, diluted in PBS) for 2 h at room temperature. Plates were blocked with 5 % skim milk in PBS containing 0.05 % Tween 20 (PBST) for 1 h at room temperature. After three washes with PBST, 100 µl of serum samples diluted two-fold in PBS (starting dilution 1:100) were added and the plates were incubated for 1 h at room temperature. Bound antibodies were detected after three washes with PBST using horseradish peroxidase (HRP)-conjugated anti-Syrian hamster IgG (Abcam) diluted 1:2500 in blocking buffer. Following incubation for 1 h at room temperature, plates were washed three times with PBST and bound HRP was detected using the ABTS Peroxidase Substrate System (SeraCare). The absorbance was measured at 405 nm; sera were considered positive when absorbance was higher than three standard deviations above the mean of negative control sera.

2.10. Statistics

Statistical analysis was performed in Prism 10 (GraphPad). When multiple groups were compared to a PBS-treated control group, including leukocyte counts, histology scores, viral loads in tissues and swabs, and ELISA results, statistical analysis was performed

using one-way ANOVA with Dunnett's post-test. For analyses where only two groups were compared, including viral loads in tissues or swabs, the Mann-Whitney test with Bonferroni Dunn correction was used. For survival data, the log-rank (Mantel-Cox) test was used. P-values<0.05 were considered statistically significant.

3. Results

3.1. Dexamethasone treatment produces leukogram shifts in syrian hamsters

To determine if dexamethasone produces similar changes in the leukogram as those observed in humans and other species, we treated Syrian hamsters for 5 days with a dose of dexamethasone considered anti-inflammatory (0.2 mg/kg) or immunosuppressive (2 mg/kg) once daily for 5 days. Two days after the last treatment, we collected blood and performed a complete blood cell count (Fig. 1). Compared to PBS treated animals, those receiving dexamethasone exhibited a dose-dependent increase, though not statistically significant, in circulating neutrophils (count and percent of total leukocytes). A similar dose-dependent decrease was observed in total lymphocyte numbers, with 2 mg/kg dexamethasone producing a statistically significant decrease ($p = 0.0017$). This is a typical leukogram shift, seen with exposure to endogenous or exogenous corticosteroids, often called a steroid leukogram (Stockham and Scott, 2008). Based on these findings, we were confident that dexamethasone exerts similar effects on hamsters as it does in other species.

3.2. Reduced pulmonary pathology NiV-infected hamsters treated with an anti-inflammatory dose of dexamethasone

We tested the effect of the anti-inflammatory and immunosuppressive doses of dexamethasone on the outcome of NiV infection. Due to the importance of timing of treatment initiation in COVID-19 patients receiving dexamethasone (RECOVERY Collaborative Group et al., 2021), treatment was initiated at 2 or 4 dpi, and continued for 5 days. Clinical signs observed in animals that became severely ill and/or reached endpoint criteria were similar in all groups, regardless of treatment, and matched those previously described in hamsters inoculated with NiV (DeBuysscher et al., 2013). Respiratory signs (tachypnea, dyspnea), neurological signs (ataxia, seizures, torticollis, and paresis), and nonspecific signs of illness (rapid weight loss, lethargy) were observed. All of the animals that received an immunosuppressive dose of dexamethasone reached endpoint criteria, regardless of treatment initiation time (Fig. 2A). Animals treated with the anti-inflammatory dose of dexamethasone on 2–6 dpi also all reached endpoint criteria, while two animals treated on 4–8 dpi survived. One of the PBS-treated control animals also survived (Fig. 2A). Lungs and brains were collected from animals that reached endpoint criteria or survived to end of study and analyzed for viral loads and histologic changes. No differences in viral loads were observed in the brain or lungs in any of the groups when compared with PBS-treated animals (Fig. 2B). On histologic exam, the animals in the group that received the anti-inflammatory dose starting at 4 dpi did not have any pulmonary lesions, nor was Nipah viral antigen detected in the lungs of these animals (Fig. 2C). Severe pulmonary pathology and viral antigen was observed in both groups treated with an immunosuppressive dose (Fig. 2C). IHC positivity in the brain was observed in moderate to high amounts in all groups except for the anti-inflammatory group with treatment

starting at 4 dpi, where only one animal exhibited a small focal region of positivity (Fig. 2C). Histopathologic features were similar between all groups, when they were observed and were similar to those described previously in NiV-infected hamsters (DeBuysscher et al., 2013; Munster et al., 2012). Mild pulmonary lesions were typically characterized by type II pneumocyte hyperplasia and small aggregates of lymphocytes and macrophages within the interstitium, and generally contain scant or no Nipah viral antigen (Fig. 3). Severe pulmonary lesions were characterized by necrotizing vasculitis, fibrin exudation, hemorrhage, pulmonary edema, infiltrating leukocytes, and syncytia formation (Fig. 3). These regions of severe pulmonary pathology were typically associated with abundant viral antigen. In the brain, severe lesions were distributed throughout the brain and were characterized by non-suppurative meningoencephalitis, malacia, edema, vasculitis, and glial nodules with variable NiV immunoreactivity (Fig. 3). Moderate to large foci of Nipah viral antigen may be observed in the brain, including in neurons, with no or very minimal pathologic changes (Fig. 3).

Given that treatment of NiV-M-infected hamsters with an anti-inflammatory dose of 0.2 mg/kg dexamethasone on 4–8 dpi appeared to result in an albeit statistically non-significant absence of pulmonary pathology and slightly increased survival compared to other treatment groups, a second experiment was performed with larger groups of animals (n = 10/group) to improve statistical power. In this experiment, treatment was extended from 5 to 10 days, in an effort to treat during the window of rapid mortality observed in the previous experiment (Fig. 2A). Two groups of 14 Syrian hamsters were inoculated intranasally with NiV-M and treated with 0.2 mg/kg dexamethasone or an equivalent volume of PBS on 4–13 dpi. On 6 dpi, 4 animals from each group were euthanized to analyze viral loads and histopathologic changes in tissues. There was no difference in survival between dexamethasone-treated and control animals (Fig. 4A). Clinical signs were similar to those described above and no differences in clinical manifestations were observed between the treatment groups. No difference in viral loads was observed in the brain or lungs on 6 dpi between the two groups (Fig. 4B), nor in animals that either met endpoint criteria or survived to end of study (Fig. S1A). Nasal and oropharyngeal swabs were collected on 1–11 dpi to assess whether dexamethasone treatment resulted in increased virus shedding and thus a potential increased risk of virus transmission under treatment. There was no difference in virus shedding between dexamethasone-treated and control groups (Fig. 4C). Together, viral loads in swabs and tissue indicate that the anti-inflammatory dose of dexamethasone does not result in increased virus replication.

Despite a lack of increased survival in dexamethasone-treated animals, pulmonary pathology and the presence of viral antigen were ~3-fold lower in the dexamethasone-treated group compared to PBS-treated animals (Fig. S1B). Although this finding was not statistically significant, it may suggest an effect of dexamethasone treatment on pulmonary disease.

3.3. Remdesivir treatment increases survival

We wanted to determine if the non-significant reduction in pulmonary pathology in NiV-infected hamsters treated with dexamethasone could be of clinical benefit when combined with an antiviral drug. Remdesivir is licensed for use in COVID-19 patients and has been

shown to protect African green monkeys from lethal NiV infection when administered early after virus inoculation (Lo et al., 2019). Additionally, remdesivir has been administered to Nipah virus disease patients in India (As et al., 2024). To determine the efficacy of combined treatment with dexamethasone and remdesivir, we first set out to find a remdesivir dose resulting in 40–60 % survival, so that any change in survival when dexamethasone treatment was added could be detected. Three groups of 10 Syrian hamsters were inoculated with NiV-M and received 20 mg/kg remdesivir, 25 mg/kg remdesivir, or an equivalent volume of vehicle intraperitoneally once daily on 1–7 dpi. Remdesivir-treated animals that met endpoint criteria exhibited clinical signs similar to those described above, including respiratory, neurologic, and nonspecific clinical signs. Treatment with 25 mg/kg remdesivir resulted in 60 % survival in NiV-infected hamsters (Fig. 5). Treatment with remdesivir did not alter virus shedding in nasal or oropharyngeal swabs (Fig. S2A), similar to previous observations in African green monkeys (Lo et al., 2019). Additionally, there was no difference in viral loads in the brain or lung of two groups of 4 additional hamsters treated with 20 or 25 mg/kg remdesivir and euthanized on 6 dpi (Fig. S2B), or between remdesivir-treated animals and vehicle-treated animals that reached endpoint criteria or end of study (Fig. S2C).

3.4. Combined dexamethasone and remdesivir treatment does not improve survival in NiV-infected hamsters

Next, we assessed the effect of combined dexamethasone and remdesivir treatment in NiV-M-inoculated Syrian hamsters. Four groups of 10 hamsters were inoculated with NiV-M and treated with 0.2 mg/kg dexamethasone on 4–13 dpi, 25 mg/kg remdesivir on 1–7 dpi, 0.2 mg/kg dexamethasone on 4–13 dpi and 25 mg/kg remdesivir on 1–7 dpi, or equivalent volumes of PBS and vehicle. In the group treated with 25 mg/kg remdesivir, we observed 40 % survival, while only 20 % of animals treated with remdesivir and dexamethasone survived (Fig. 6A), indicating a potential loss of clinical benefit with combined treatment. In all groups, clinical signs were similar to those described above, regardless of treatment. Four additional hamsters were included in each group to assess viral loads and histopathologic changes on 6 dpi. There were no statistically significant differences in viral loads in lungs and brains of animals treated with remdesivir and/or dexamethasone as compared to control animals (Fig. 6B). Likewise, there was no difference in viral loads in the brain or lung of animals that reached endpoint criteria or planned end of study (Fig. S3A). There was no statistically significant difference in virus shedding in nasal and oropharyngeal swabs in any of the groups when compared to animals treated with PBS and vehicle (Fig. 6C). Although not statistically significant, animals treated with dexamethasone had fewer pathologic lesions in the lungs than any other treatment group (Fig. S3B). Similarly, the dexamethasone treatment group did not have any detectable NiV antigen in the lung (Fig. S3B). In all groups, lesions and viral antigen were a frequent finding in the CNS. Histologic findings in the brain were similar to those described above, characterized by non-suppurative meningoencephalitis, malacia, edema, vasculitis, and glial nodules (Fig. 3).

3.5. Dexamethasone and remdesivir treatment affect the proinflammatory response to NiV infection in the lungs

To assess the effect of dexamethasone and remdesivir treatment on the proinflammatory response in the lungs and how this relates to the observed changes in survival (Fig. 6A), we performed RT-qPCR to detect expression of IFN-γ, IL-1β, IL-6, TNF-α, and RIG-I on lungs collected from animals at endpoint (i.e. when animals reached endpoint criteria or 28 dpi). Compared to control animals treated with PBS and vehicle, expression of IL-1β, IL-6, TNF-α, and RIG-I was statistically significantly reduced in the lungs of animals treated with dexamethasone, in line with the observed reduction in pulmonary lesions (Fig. 6D). Similarly, remdesivir treatment resulted in a significant reduction in expression of IL-1β, TNF-α, and RIG-I. By contrast, combined treatment with dexamethasone and remdesivir did not result in a statistically significant reduction of expression of proinflammatory cytokines; rather, expression of IFN-γ and IL-6 increased, although only increased expression of IL-6 was statistically significant (Fig. 6D). Thus, the changes in expression of proinflammatory cytokines in the lungs correlate with the presence or absence of lung lesions (Fig. S3B) and decreased survival observed with combined treatment versus treatment with remdesivir alone.

3.6. Dexamethasone treatment does not affect the humoral response to NiV infection

Dexamethasone reduced pulmonary lesions (Fig. S4) but did not increase survival. To ensure that dexamethasone did not interfere with the adaptive immune response to NiV, specifically the generation of NiV-specific antibodies, anti-NiV-G IgG antibodies were quantified by ELISA in sera from animals that met endpoint criteria or planned end of study (Fig. 6E). All animals seroconverted, and there was no statistically significant difference in IgG titer between groups treated with dexamethasone and/or remdesivir as compared to the control group (Fig. 6E), indicating that the dexamethasone treatment did not impair antibody generation.

4. Discussion

As an emerging, highly pathogenic virus that affects people in low to middle income countries, affordable and accessible treatments against NiV are critically needed. Here, we investigated the therapeutic potential of dexamethasone. Dexamethasone has been utilized in a number of inflammatory conditions, including infectious diseases such as bacterial meningitis (Heckenberg et al., 2014), non-bacterial encephalitis (Di Flumeri et al., 2024), and Epstein-Barr virus infection (Liang et al., 2024). However, the effect of dexamethasone treatment in the case of viral infections in general is highly variable. In the case of COVID-19, treatment with dexamethasone is beneficial at a specific point in disease, and if administered too early can result in worse outcomes (Bi et al., 2020; Liu et al., 2024). In other respiratory viral infections such as influenza A pneumonia, dexamethasone treatment is associated with higher mortality (Ni et al., 2019). The reasons for the highly variable effects of dexamethasone in viral pneumonias are likely due to differences in the pathophysiology of different viruses and the extensive effects glucocorticoids can have, both on the immune system and tissues affected by the virus. We felt that assessing the role of dexamethasone in NiV infection was important as it is so widely available and may be a

drug clinicians employ in cases of severe, life-threatening respiratory disease or encephalitis when antiviral therapies are unavailable or insufficient.

Some animals throughout our experiments met endpoint criteria and were euthanized with no significant lesions in the lungs or brain, regardless of treatment group. In these animals, viral RNA was typically found in tissues and, in the final combination treatment experiment, seroconverted, confirming they were infected with NiV-M. This suggests that NiV infection can lead to severe disease without severe pulmonary or brain lesions. Lesions elsewhere in the brain and lung cannot be ruled out as only one histologic section is examined from both organs; however, this section is deliberately taken from the center of the formalin-fixed paraffin embedded tissue, making it unlikely that pulmonary lesions elsewhere would be severe enough to cause respiratory distress. We suspect that vasculitis, a hallmark of NiV infection, contributes to a form of inflammatory systemic shock, leading to multisystem dysfunction and rapid decompensation before histologic lesions have time to develop in some animals. This phenomenon has been described in other viral illnesses, such Dengue shock syndrome (Wang et al., 2020), Ebola virus disease (Letafati et al., 2023), and influenza (Gu et al., 2021).

While we found that dexamethasone treatment reduced expression of proinfammatory cytokines in the lungs and pulmonary lesions in NiV-infected animals, there was no effect on survival. Off-label use of remdesivir in NiV disease patients has been described (As et al., 2024), leading us to explore the effect of dexamethasone and remdesivir treatment. The addition of remdesivir to dexamethasone treatment did not improve clinical outcome. In fact, animals that received combined treatment succumbed to disease at the same rate and frequency as control-treated animals, while remdesivir alone improved survival. The anti-inflammatory effect of dexamethasone as measured by the expression of proinflammatory cytokines and the interferon-stimulated gene RIG-I, observed in the lungs after dexamethasone treatment disappeared when animals were treated with dexamethasone and remdesivir. A potential explanation for this effect is that dexamethasone may be inducing a drug-drug interaction with remdesivir and negatively affect its efficacy. These drug-drug interactions are often understudied, and our study demonstrates the importance of evaluating these potential interactions prior to and during administration of combined treatments to human patients (Jacobs et al., 2022). In vitro, dexamethasone does not affect the hydrolysis of remdesivir, meaning that dexamethasone does not change the metabolization of remdesivir into its active form (Zhang et al., 2022). Combination treatment with dexamethasone and remdesivir has been the standard of care in hospitalized COVID-19 patients on supplemental oxygen in the US (COVID-19 Treatment Guidelines Panel, 2024; Infectious Diseases Society of America, 2024) and this combination treatment resulted in improved survival (Mozaffari et al., 2025). In vitro and in COVID-19 patients, addition of dexamethasone reduced remdesivir-induced liver toxicity (Liu et al., 2023) implying a drug-drug interaction between the two occurs. Dexamethasone is known to induce cytochrome P450 3A4 (CYP3A4), a common enzyme associated with drug metabolism (McCune et al., 2000). Induction of this enzyme can alter how rapidly a drug is metabolized and decrease its efficacy. Remdesivir has also been found to have drug-drug interaction potential, specifically with CYP3A4 substrates (Peng et al., 2023). However, the fact that this negative interaction between dexamethasone and remdesivir was

not observed in COVID-19 patients implies that the effect is either virus specific, specific to Syrian hamsters, or the timing of dexamethasone administration needs to be adapted when infection dynamics change under remdesivir treatment. Increasing the frequency of treatment administration of dexamethasone and remdesivir could result in more constant levels of these drugs and might potentially counteract the unexpected outcome of the combined treatment.

One limitation of our study is that we could not investigate more drug combinations and timings of administration. Although we investigated two doses of dexamethasone at two treatment initiation time points and two concentrations of remdesivir prior to testing the effect of the combination treatment, we did not test additional combinations after finding that dexamethasone negates the beneficial effect of remdesivir. Additional studies with alternative treatment doses, schedules, or in combination with other NiV-targeting drugs may yield different results.

Taken together, our data caution against the use of dexamethasone treatment in NiV disease patients in combination with other antiviral treatments when no preclinical efficacy data are available supporting the combined treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data included in this manuscript are available in Figshare at: 10.6084/m9.figshare.28821623.

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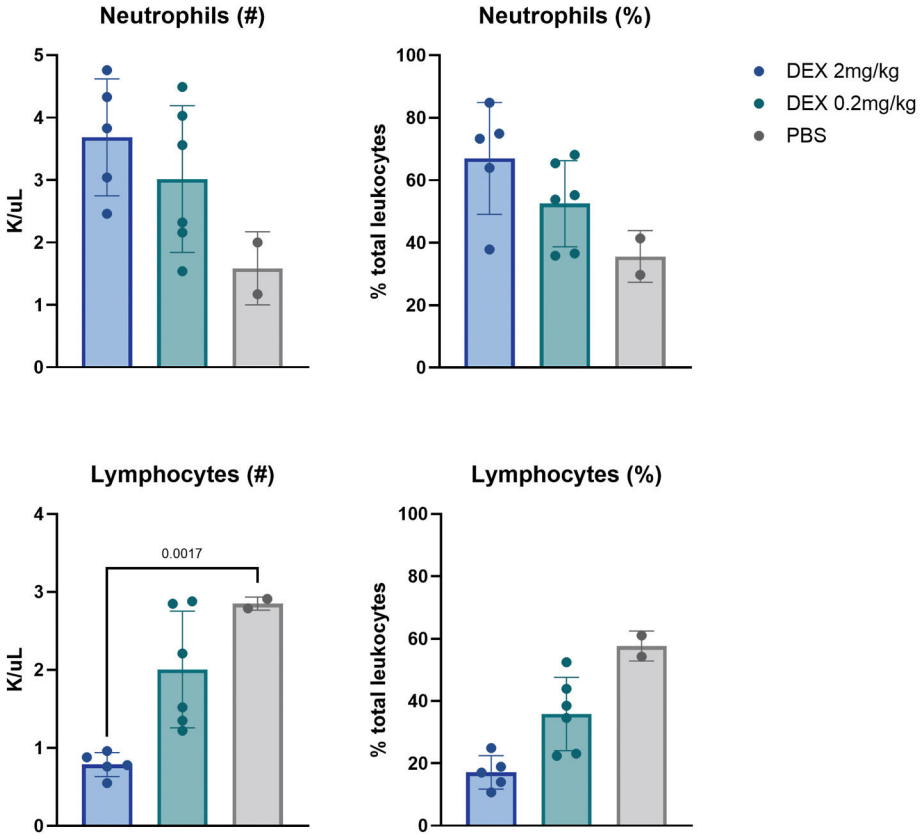

Fig. 1. Dexamethasone treatment in Syrian hamsters results in a dose dependent neutrophilia and lymphopenia, consistent with a steroid leukogram.

Two groups of 6 hamsters were treated with an anti-inflammatory (0.2 mg/kg) or an immunosuppressive (2 mg/kg) dose of dexamethasone (DEX), and one group of 2 hamsters was treated with an equivalent volume of PBS. Animals were treated once daily for 5 days; blood was collected two days after the last treatment. Neutrophil and lymphocyte cell counts and percent of total leukocytes were determined using a Procyte DX. Statistical analysis was conducted using one-way ANOVA with Dunnett's post-test; P-values <0.05 are shown.

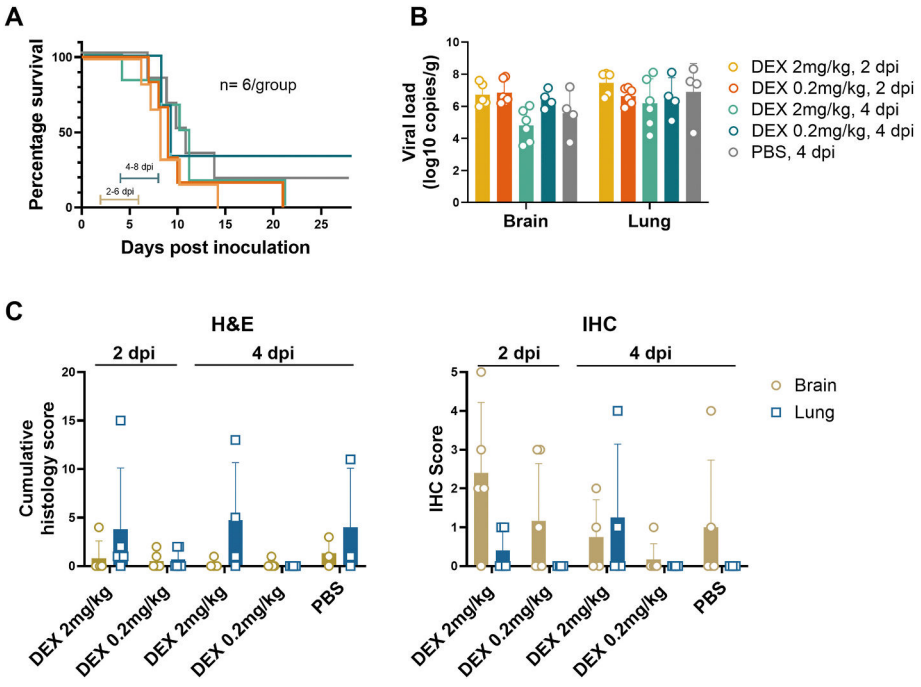

Fig. 2. Dexamethasone treatment appears to reduce pulmonary pathology in hamsters treated with an anti-inflammatory dose of dexamethasone.

Five groups of 6 hamsters were inoculated intranasally with 10^6 TCID50 NiV-M, then treated with 0.2 mg/kg dexamethasone on 2–6 dpi, 0.2 mg/kg dexamethasone on 4–8 dpi, 2 mg/kg dexamethasone on 2–6 dpi, 2 mg/kg dexamethasone on 4–8 dpi or with an equivalent volume of PBS on 4–8 dpi. (A) The percentage surviving animals over time is shown. Horizontal bars indicate treatment windows. (B) When animals met endpoint criteria or at end of study, brain and lung were collected and viral loads determined. (C) Histologic lesions and anti-NiV N immunohistochemistry (IHC) were assessed by a board-certified veterinary pathologist. For H&E-stained sections, histology scores were assigned for various pathologic features and added together for a cumulative score. For IHC sections, one score was assigned for the brain and lung respectively. Error bars indicate geometric mean ± SD (B) and mean ± SD (C). Statistical analysis was conducted using Log-rank (Mantel-Cox) test (A), or one-way ANOVA with Dunnett's post-test (B, C); p-values <0.05 are shown.

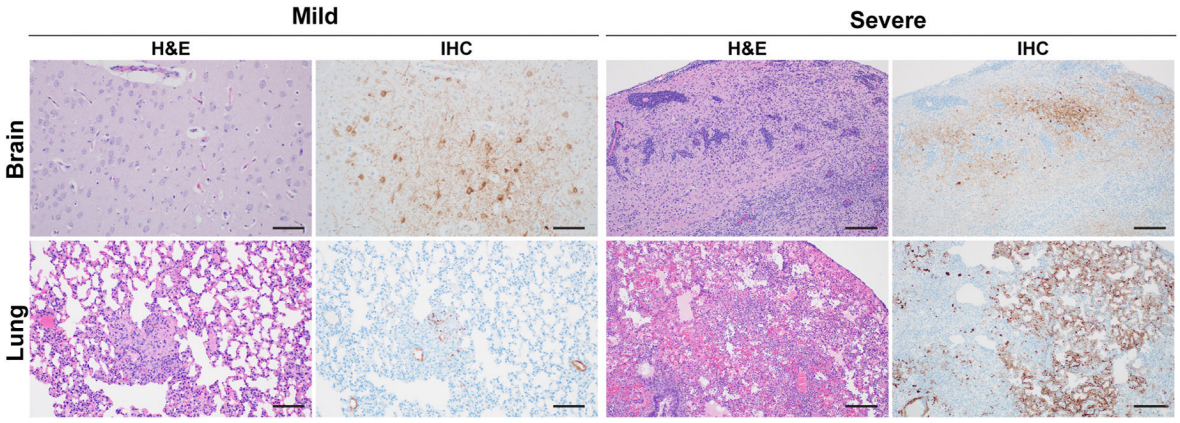

Fig. 3. Histologic lesions and viral antigen in the brain and lungs of Nipah virus-infected Syrian hamsters.

Representative mild and severe histologic and immunohistochemical findings associated with NiV infection in the brain and lungs of hamsters inoculated intranasally with 10^6 TCID50 NiV-M are shown. Representative lung and brain images were chosen from different animals. In the brain (top panels), mild lesions are characterized by occasional neuronal necrosis and small regions of edema. On IHC, mild histologic lesions can exhibit abundant NiV antigen, typically within neurons. Severe lesions in the brain are characterized by marked non-suppurative meningoencephalitis, edema, malacia, and glial nodules. NiV antigen is often abundant in severe lesions in the brain. In the lung (bottom panels), mild lesions are characterized by small peribronchiolar or interstitial aggregates of lymphocytes, and NiV antigen is often scant. Severe pulmonary lesions are characterized by necrotizing vasculitis, hemorrhage, fibrin exudation, edema, and infiltrating leukocytes. NiV antigen is abundant in severe lung lesions. Mild lesions: scale bar = 100 μm. Severe lesions: scale bar = 200 μm.

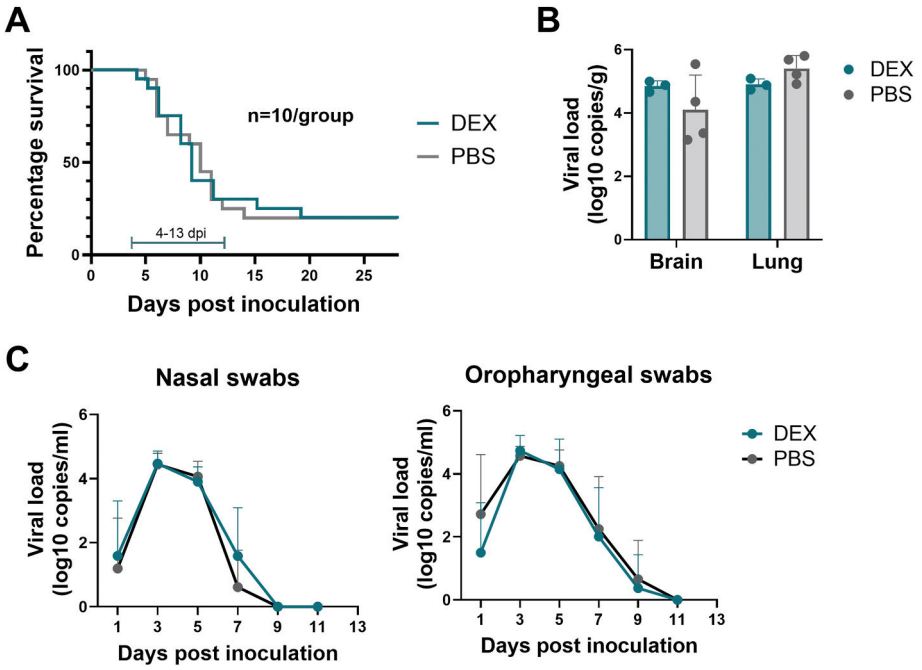

Fig. 4. An anti-inflammatory dose of dexamethasone does not increase survival in Nipah virus-infected Syrian hamsters.

Two groups of 10 animals were inoculated intranasally with 10^6 TCID50 NiV-M and treated with 0.2 mg/kg dexamethasone (DEX) or an equivalent volume of PBS on 4–13 dpi. (A) The percentage of surviving animals is shown over time. The horizontal bar indicates the treatment window. (B) Two additional groups of 4 hamsters were treated as in A, and euthanized at 6 dpi. Tissues were collected and viral loads determined. Data are plotted as geometric mean ± SD. (C) Nasal and oropharyngeal swabs were collected from animals in A on 1, 3, 5, 7, 9, and 11 dpi. Data are plotted as geometric mean +SD. Statistical analysis was conducted using Log-rank (Mantel-Cox) test (A), Mann-Whitney (B), and Mann-Whitney with Bonferroni Dunn correction (C); p-values <0.05 are shown.

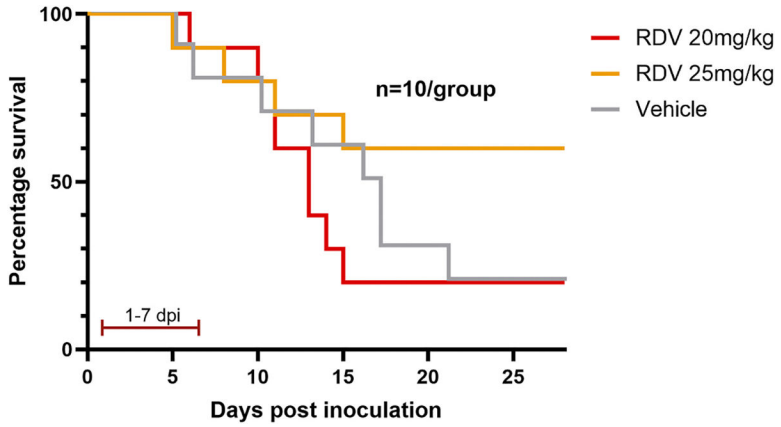

Fig. 5. Remdesivir treatment improves survival in Nipah virus-infected Syrian hamsters.

Three groups of 10 animals were inoculated intranasally with 10^6 TCID50 NiV-M, then treated with 20 mg/kg remdesivir, 25 mg/kg remdesivir (RDV), or an equivalent volume of vehicle on 1–7 dpi. The percentage of surviving animals is shown over time. The horizontal bar indicates the treatment window. Statistical analysis was conducted using Log-rank (Mantel-Cox) test; p-values <0.05 are shown.

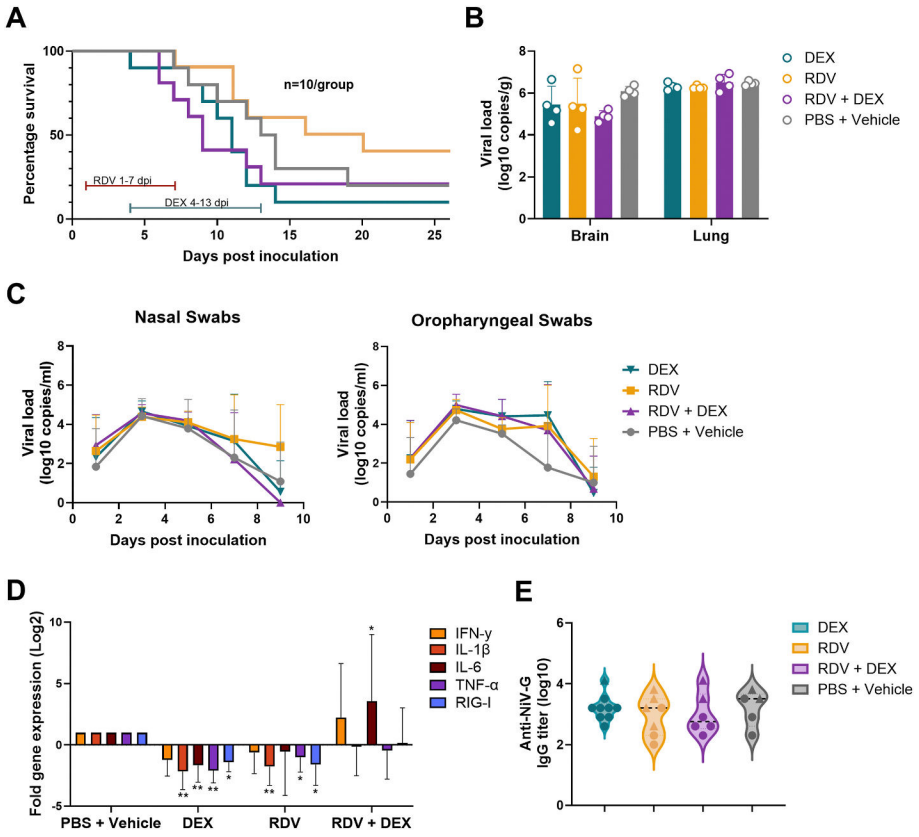

Fig. 6. Combined remdesivir and dexamethasone treatment is not beneficial in Nipah virus-infected Syrian hamsters.

Four groups of 10 Syrian hamsters were inoculated intranasally with 10^6 TCID50 NiV-M and treated with 0.2 mg/kg dexamethasone (DEX) on 4–13 dpi, 25 mg/kg remdesivir (RDV) on 1–7 dpi, 0.2 mg/kg dexamethasone on 4–13 dpi and 25 mg/kg remdesivir on 1–7 dpi, or equivalent volumes of PBS on 4–13 dpi and of vehicle on 1–7 dpi. (A) The percentage of surviving animals is shown over time. The red bar indicates the treatment duration with remdesivir/vehicle and the blue bar indicates treatment with dexamethasone/PBS. (B) Four additional groups of 4 hamsters were treated as in A, and euthanized on 6 dpi. Lung and brain tissue were collected and Nipah viral loads determined. (C) Nasal and oropharyngeal swabs were collected and analyzed for the presence of viral RNA. Data are plotted as geometric mean +SD. (D) Expression of pro-inflammatory cytokines IFN-γ, IL-1β, IL-6, and TNFα and interferon-stimulated gene RIG-I was quantified. RNA was extracted from lungs collected from animals that reached endpoint criteria or the end of the experiment on 28 dpi RT-qPCR was performed. Log2 fold change in expression was calculated using the $2^{-\Delta Ct}$ method; data were normalized to the expression detected in the control group treated with PBS and vehicle. Due to the inability to detect expression of some genes in some animals, the mean ± SD of 3–9 replicates is shown. (E) ELISA was performed to detect anti-NiV-G IgG in sera collected from animals that met endpoint criteria or planned end of study. Circles indicate animals that reached endpoint criteria, triangles indicate animals that survived to end of study. Statistical analysis was conducted using Log-rank (Mantel-Cox) test (A), one-way ANOVA with Dunnett's post-test (C, E), or two-way ANOVA with

multiple comparison correction (D); p-values <0.05 are shown. *p < 0.05; **p < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)