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EIDD-2801 resists to infection and co-infection of SARS-CoV-2 and influenza virus

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Abstract

Background The coronavirus disease 2019 (COVID-19) pandemic has exerted a catastrophic impact on public health. Meanwhile, the seasonal influenza outbreak overlaps with the current pandemic wave. There is still an urgent need to develop effective therapeutic agents for the treatment of co-infection of multiple respiratory viruses. This study aimed to investigate antiviral effects of EIDD-2801, an orally bioavailable ribonucleoside analog, and its potent therapeutic effects in co-infection of multiple respiratory viruses.

Methods BALB/c mice and hamsters were infected with IFV or SARS-CoV-2, then were dosed orally with EIDD-2801 to measure the antiviral effects of EIDD-2801. Viral replication and mRNA transcription were evaluated by quantitative polymerase chain reaction (qPCR) and protein expression by Western Blot. Influenza viral titer was assessed using EID_{50} assay.

Results EIDD-2801 was found to be significantly effective against influenza A virus and influenza B virus. The antiviral activity against SARS-CoV-2 and further co-infection with influenza virus was also distinct. EIDD-2801 had potent antiviral effects against multiple respiratory viruses both in vitro and in vivo.

Conclusion This study demonstrated that the small-molecule compound EIDD-2801, an orally available broad-spectrum antiviral agent, significantly inhibited the infection of influenza virus and SARS-CoV-2 and effectively protected animals from lethal influenza virus co-infection.

Keywords Antiviral activity, Respiratory viruses, Influenza, SARS-CoV-2, EIDD-2801, Co-infection.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed significant threats to public health and economic development worldwide [1]. Most infected patients show pneumonia symptoms ranging from respiratory complications to acute respiratory distress syndrome and multiple organ dysfunction [2]. These symptoms are associated with the respiratory system, resulting in the impairment of lung function [3–5]. Vaccines are widely available in disease prevention. However, the emergence of variants of concern (VOCs) including B.1.1.7 (alpha) [6], B.1.351 (beta) [7], P.1 (gamma) [8],

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B.1.617.2 (delta) [9] and B1.1529 (omicron) [10] lineages, characterized by enhanced transmissibility and immune evasion capabilities, poses significant risks of exacerbating pandemic trajectories [11–13]. More importantly, the co-circulation of multiple viruses [14, 15] not only exacerbates the clinical severity of co-infections but also heightens concerns regarding potential cross-transmission risks [16, 17].

Influenza virus, a highly contagious respiratory virus, also can cause respiratory complications and co-infection with other pathogens [18–20]. Both influenza virus and SARS-CoV-2 are airborne transmitted viruses following similar transmission routes [21–23]. They infect the upper and lower respiratory tracts and have the same target cells despite the different cellular receptors [24, 25]. Previous clinical studies suggested that co-infection of Influenza A virus and other respiratory viruses, including SARS-CoV-2 and other circulating influenza virus strains resulted in more severe disease and higher fatality [26–28]. Thus, recurring influenza seasons during the current pandemic potentially make the threats to public health even more critical. In the context of influenza and COVID-19 outbreaks overlap in many regions, difficulties in disease management call for the development of broad-spectrum antiviral agents for the control of co-infection of emerging viruses.

EIDD-2801 (MK-4482), an orally available pro-drug of N4-hydroxycytidine (NHC), exhibits antiviral activities against influenza virus [29, 30] and SARS-CoV-2 [31–35] in vitro and in vivo. As a cytidine analogue, EIDD-2801 inhibits viral RNA-dependent RNA polymerase (RdRp) activity by inducing random low-frequency C-U and G-A transitions, resulting in deleterious mutations and inhibition of viral RNA replication [36–38].

In this study, we evaluated the effects of EIDD-2801 against a panel of mouse-adapted viral strains, including influenza A virus (IAV), influenza B virus (IBV) and SARS-CoV-2. The results indicated that therapeutic administration of EIDD-2801 dramatically reduced viral load of divergent influenza virus and SARS-CoV-2 in upper respiratory tract and lung. Notably, oral administration of EIDD-2801 exhibits significant antiviral potency for the treatment of influenza virus and SARS-CoV-2 mono-infection and co-infection.

Materials and methods

Cells, viruses and compounds

MDCK, Vero-E6, and Huh7 cell lines used in the experiments were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, US) supplemented with 10% fetal bovine serum (FBS) (Gibco, US) and 100 U/mL penicillin-streptomycin at 37 ℃ in 5% CO_2. Influenza A virus H1N1-UI182 strain and H3N2 strain, Influenza B virus IBV/S9-E2 strain, and IBV/S9-MD strains were saved as previously described [39]. All SARS-CoV-2 strains used in this study were isolated and stored by Changchun Veterinary Research Institute according to biosafety norms. Oseltamivir phosphate, zanamivir, and baloxavir were purchased from Med Chem Express (Shanghai, CN) and EIDD-2801 was a gift from Prof. Wu Zhong [40]. All compounds were dissolved in phosphate-buffered saline (PBS) and stored at -20 ℃. All working standards were diluted in DMEM or PBS from stock solution within 1 day before the experiment and stored at 4 °C for a maximum of 6 days.

Immunofluorescence assay

MDCK cells were seeded in 48-well plates and infected with H1N1-UI182 (MOI = 0.05), IAV/H3N2 (MOI = 0.01) or IBV/S9-E2 (MOI = 0.05) respectively at 37 °C for 1 h when cell confluence reached 70–90%, then the cells were further incubated with DMEM with 2% FBS and 20 μM EIDD-2801. At 48 h post-infection (hpi), the cells were washed three times with PBS and fixed with 4% paraformaldehyde (PFA) for 20 min. After that, the cells were infiltrated with 0.2% Triton-X100 and blocked in 2% Bovine Serum Albumin (BSA) dissolved in Phosphate-buffered saline with tween (PBST) for 1 h. Anti- IAV NP antibody (Abcam, ab128193, Shanghai, China, 1:200) or Anti-IBV NP antibody (GeneTex, GTX636194, CA, USA, 1:1000) was added respectively and incubated with cells overnight at 4 ℃. After the primary antibodies, cells were washed three times using PBS and incubated with Alexa Fluor 488-labeled goat anti-Rabbit IgG antibody(Abcam, ab150077, Shanghai, China) for 2 h without light. After nuclei staining with Hoechst (Thermo Fisher Scientific, H3569, 1 μg/mL) for 10 min, fluorescent images were taken with a fluorescence microscope (Carl Zeiss, Germany) and the cell number was quantified using Image-J software.

In vitro infection experiment of SARS-CoV-2

Vero-E6 and Huh7 cells were pre-seeded in 96-well plates at the density of 8000 cells/well. When cell confluence reached 70–90%, cells were infected with diluted SARS-CoV-2 variants (BJ01, Delta, BA.2, BF.7 and XBB.1.9.1) at a multiplicity of infection (MOI) of 0.01 for 1 h. Following viral adsorption, a concentration gradient of EIDD-2801 (0.003, 0.03, 0.3, 3, 30 and 60 μM) was administered and maintained under standard culture conditions (37 °C, 5% CO_2) for 36 h. Then 200 μL of supernatant in each group was collected to detect viral RNA copy numbers using qRT-PCR.

Quantitative real-time PCR (qRT-PCR)

200 μL cell supernatants or supernatants from the lysed tissues were harvested for RNA extraction (TIANGEN, YDP804-T1) and RNA copies were quantified

by qRT-PCR according to the manufacturer's instructions. The primers designed to amplify the SARS-CoV-2 *ORFlab* gene were as follows:

Forward sequence: 5′-CCCTGTGGGTTTTACACTTAA-3′.

Reverse sequence: 5′-ACGATTGTGCATCAGCTGA-3′.

Probe sequence: 5′-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3′.

The primers designed to amplify the H1N1 *M* gene were as follows:

Forward sequence: 5′-GTCTTCTAACCGAGGTCGAAA-3′.

Reverse sequence: 5′-AAGATCTGTGTTCTTTCCTGCAAA-3′.

Probe sequence: 5′-FAM-CCCTCAAAGCCGAGATCGC-TAMRA-3′.

In vivo infection experiment of influenza viruses in mouse model

Six to eight-week-old female BALB/c mice were purchased from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) and were quarantine-housed in individually ventilated cages. Then the mice were randomly assigned into 3 groups, including blank control (Control), virus control (Virus), and drug-treated group (Virus + Drug), with 12 mice per group. All mice were infected with influenza viruses by nasal drip (except the blank group), and were treated with PBS or oseltamivir phosphate (25 mg/kg/day, orally), zanamivir (5 mg/kg/day, intraperitoneally), baloxavir acid (5 mg/kg/day, subcutaneously) and EIDD-2801 (500 mg/kg/day, orally) for 5 days. For mono-infection of influenza virus, mice were intranasally infected with 50 μL viral dilutions ($10\times MLD_{50}$ of H1N1-UI182 or $1\times MLD_{50}$ of IBV/S9-MD) separately. For co-infection, mice were intranasally infected with 50 μL viral dilutions of $1\times MLD_{50}$ H1N1-UI182 and $0.1\times MLD_{50}$ of IBV/S9-MD. Body weight changes and survival status were monitored daily for 14 days. Mice with more than a 25% decrease in body weight were euthanized humanely. At 5 days post-infection (dpi), 5 mice were randomly selected from each group and euthanized by exsanguination under deep anesthetization, and a part of the organs was collected and fixed with 4% paraformaldehyde (PFA) for pathological analysis. The other part collected lungs was immersed in Radio Immunoprecipitation Assay Lysis (RIPA) buffer (Beyotime, China) with protease inhibitor (MCE, HY-K0011, Shanghai, China) for Western Blot (WB) or in DMEM for quantification of the viral titer.

Co-infection experiment of SARS-CoV-2 and influenza A virus in golden hamster

4-week-old female golden hamsters were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and were intranasally inoculated with 100 μL virus dilution of SARS-CoV-2/Omicron BF.7 (2000 PFU) and H1N1-UI182 ($1\times MLD_{50}$). The co-infected animals were treated with vehicle (PBS) or EIDD-2801 (350 mg/kg/day, orally) for 5 days. Body weight changes were monitored for 14 days. Nasal lavage fluid was collected at 2 and 4 dpi separately. At 3 days post-infection, 4 hamsters were randomly selected from each group and euthanized by exsanguination under deep anesthetization. Tissues were harvested following euthanasia to measure lung weight and quantify viral loads.

Western blot

Lung tissues harvested from experimental animals were lysed with RIPA buffer. After protein quantification using Enhanced BCA Protein Assay Kit (Beyotime, China), lysates were mixed with 5× loading buffer (Beyotime, China) and boiled for 5 min. Lung tissue homogenates were prepared from $n = 3–5$ animals per group, and equal amounts of protein (10 μg) were loaded per lane. The proteins were transferred to a polyvinylidene difluoride (PVDF) membrane after being subjected to SDS-PAGE. The PVDF membranes was blocked in 5% BSA solution for 1 h. After blocking, the membranes were incubated with either Anti-IAV NP antibody (GeneTex, GTX125989, CA, USA, 1:5000) and Anti-IAV M antibody (GeneTex, GTX636677, CA, USA, 1:5000) or Anti-IBV NP (GeneTex, GTX636194, CA, USA, 1;5000) and Anti-IBV M antibody (GeneTex, GTX128537, CA, USA, 1:5000) overnight at 4 ℃. Anti-β-actin (Abcam, ab6276, Shanghai, China, 1:5000) or anti-GAPDH (Abcam, ab8245, Shanghai, China, 1:1000) was used as a loading control. Following three washes with Tris-buffered saline containing 0.1% Tween-20 (TBST; Beyotime, China), the membranes were incubated with species-specific secondary antibodies: HRP-conjugated goat anti-rabbit IgG (Abcam, ab205718; 1:1000) or goat anti-mouse IgG (Abcam, ab205719; 1:1000) in TBST for 1 h at room temperature (RT). Finally, the membranes were incubated by enhanced chemiluminescence (ECL) Western Blot substrate after washed another three times with TBST for the analysis using a fluorescence image analysis system (Tanon, China). Band intensities were quantified using Image-J, and results were normalized to loading controls.

Statistical analysis

All the calculated results were presented as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA or student's t-test with GraphPad Prism 9.0 software. Compared with the Virus-infected

group, the statistical significances were defined as $P < 0.05$ (*), and the higher significance was denoted by $P < 0.01$ (**) and $P < 0.001$ (***).

Results

Potent inhibitory activity and therapeutic effect of EIDD-2801 against influenza viruses in vitro and in vivo

Consistent with prior studies [29, 30, 41], EIDD-2801 demonstrated potent antiviral activity against influenza viruses in this study. In MDCK cells infected with H1N1-UI182, EIDD-2801 treatment reduced IAV NP protein expression by approximately 45% (Fig. S1A, B) and viral titers by 2.5 $\log_{10}$ compared to the control group (Fig. S1C). Similarly, for H3N2, IAV NP protein levels decreased by 96% (Fig. S1D, E) with a 2 $\log_{10}$ reduction in viral titers (Fig. S1F). Notably, EIDD-2801 also exhibited efficacy against IBV/S9-E2, achieving a 63% inhibition of NP protein expression (Fig. S1G, H) and a 1.4 $\log_{10}$ decrease in viral loads (Fig. S1I). To evaluate the in vivo antiviral efficacy of EIDD-2801 against both IAV and IBV, BALB/c mice infected with influenza virus were treated with EIDD-2801 and compared to multiple standard anti-influenza drugs. Briefly, BALB/c mice were intranasally inoculated with a lethal dose of H1N1-UI182 or a non-lethal dose of IBV/S9-MD and subsequently treated with EIDD-2801 (500 mg/kg/day) via oral gavage starting 12 h post-infection (Fig. 1A). After 5 consecutive days of treatment, EIDD-2801 administration significantly protected infected mice from weight loss in both IAV and IBV mouse models, demonstrating superior efficacy compared to oseltamivir phosphate and zanamivir (Fig. 1B, D). Unexpectedly, EIDD-2801 treatment exhibited higher protection efficacy (62.5%) than oseltamivir phosphate (50%) in H1N1-UI182-infected mice (Fig. 1C), highlighting its superior antiviral potential. Necropsy of lung tissues revealed that both IAV and IBV infections caused dark red discoloration with patchy hemorrhage on the lung surface (indicated by black arrows), accompanied by severe pathological damage and a significant increase in lung index (Fig. 1E, F). These effects were markedly alleviated by EIDD-2801 treatment. Meanwhile, Western Blot results revealed that EIDD-2801 treatment significantly

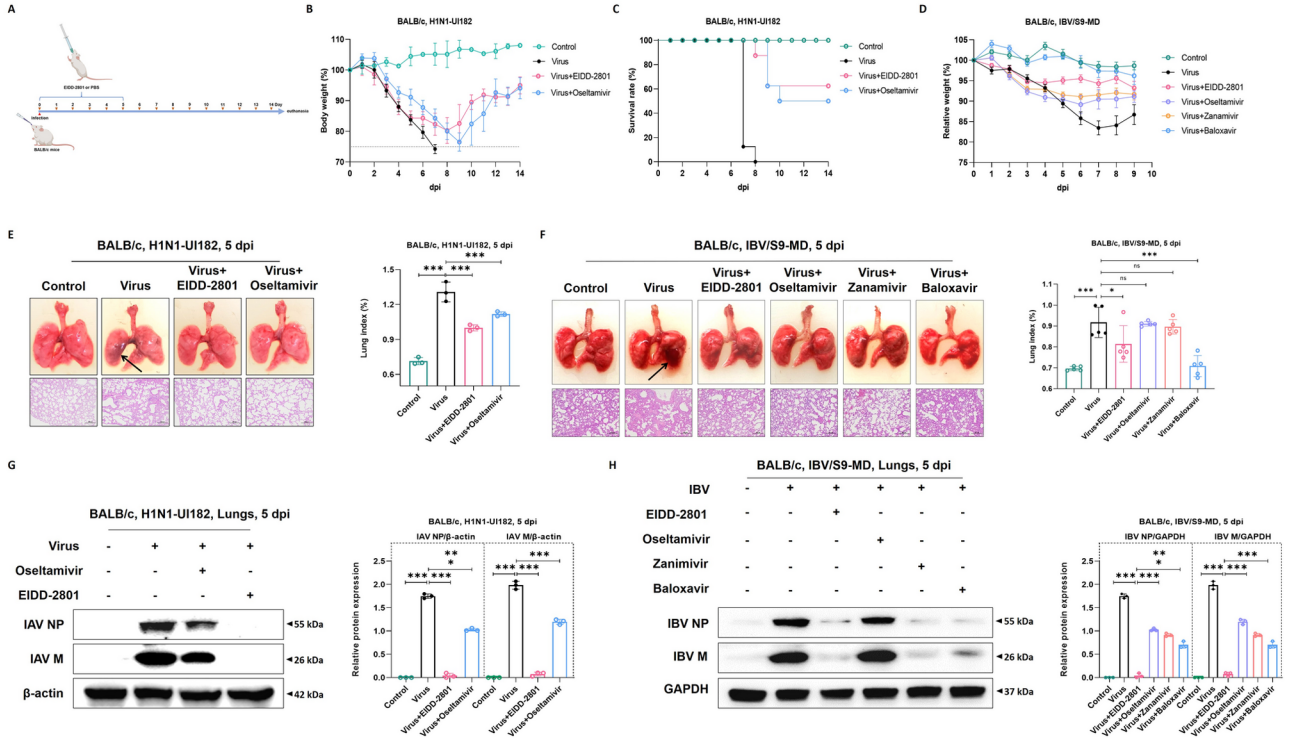

Fig. 1 Administration of EIDD-2801 effectively protects mice against lethal and non-lethal influenza virus challenge. **A** Schematic of the mouse pathogenesis study. BALB/C mice were orally administered EIDD-2801 at a dose of 500 mg/kg for up to 5 days following intranasal infection with H1N1-UI182 (10×MLD_{50}) or IBV/S9-MD (1×MLD_{50}). **B** Body weight change of mice infected with H1N1-UI182. **C** Survival curves of mice infected with H1N1-UI182. **D** Body weight change of mice infected with IBV/S9-MD. **E-F** Representative images of lung tissue are presented, with photomicrographs showing histopathological changes (scale bars = 200 μm). Hematoxylin and eosin staining (H&E) and lung index (%) in groups infected with IAV **E** or IBV/S9-MD **F**. **G** Western Blot analysis of influenza A virus nucleoprotein (IAV NP) and matrix protein (IAV M) expression in lung tissue homogenates from H1N1-UI182-infected mice ($n = 3$). **H** Western Blot analysis of influenza B virus nucleoprotein (IBV NP) and matrix protein (IBV M) expression in lung tissue homogenates from IBV/S9-MD-infected mice ($n = 3$). Band intensities were measured and the expression of β-actin or GAPDH was used as loading control. Statistically significant differences between groups were determined by the Student's *t* test (*) $0.01 < P < 0.05$, (***) $P < 0.001$, "ns" denotes no significance

inhibited viral protein expression in IAV and IBV. Compared to the viral control group, EIDD-2801 treatment significantly reduced the expression levels of IAV NP and IAV M to below the limit of detection (LOD) in mouse lungs (Fig. 1G). Similarly, for IBV-infected mice (Fig. 1H), EIDD-2801 treatment resulted in a 95% reduction in the expression levels of IBV NP and IBV M compared to the untreated control group (Fig. 1H). These data indicate that oseltamivir can still protect mice from lethal H1N1-UI182 infection; however, its effects on reducing lung damage and viral load are less pronounced. Furthermore, zanamivir failed to ameliorate IBV-induced lung damage. Collectively, the antiviral efficacy of EIDD-2801 was superior to that of both oseltamivir and zanamivir.

In vivo efficacy of EIDD-2801 against co-infection of influenza A virus and influenza B virus

To evaluate the antiviral efficacy of EIDD-2801 against co-infected with both IAV and IBV, BALB/c mice were simultaneously infected with H1N1-UI182 and IBV/S9-MD. Compared to the Virus group, mice treated with EIDD-2801 exhibited significantly reduced weight loss, surpassing even the effects observed in the oseltamivir-treated group (Fig. 2A). Furthermore, oral administration of EIDD-2801 ensured complete survival of the mice, comparable to the outcomes achieved with oseltamivir or baloxavir treatment (Fig. 2B). EIDD-2801 treatment led to a significant reduction in the expression of IAV M and IBV M in co-infected mouse lung tissues, with both proteins barely detectable on Western Blot (Fig. 2C). In addition, EIDD-2801 significantly alleviated extensive hemorrhage, inflammation, and edema in mouse lung tissues caused by co-infection with IAV and IBV (Fig. 2D). We further observed that EIDD-2801 treatment significantly reduced the lung index and histopathological scores (Fig. 2E, F). Consistent with Western Blot analysis, the viral load in mouse nasal turbinates was significantly decreased by 1.5 to 2 Log_{10} in both the EIDD-2801 and baloxavir treatment groups (Fig. 2G). These findings demonstrate that EIDD-2801 effectively protects mice against lethal mixed infections of IAV and IBV.

In vivo efficacy of EIDD-2801 against co-infection of influenza A virus and SARS-CoV-2

EIDD-2801 has been approved for clinical trials targeting SARS-CoV-2 and demonstrated strong antiviral activity against multiple variants [42–45]. Given its potent antiviral effects against influenza viruses, assessing its therapeutic potential in combating co-infections of influenza A virus and SARS-CoV-2 is of great importance. Firstly, in vitro experiments demonstrated that EIDD-2801 significantly reduced the viral load of multiple variants in a dose-dependent manner in the supernatants of Vero-E6 (Fig. S2A) and Huh7 (Fig. S2B) cells, These variants included isolated BJ01 strain, Delta variant, and Omicron variants (BA.2, BF.7, and XBB.1.9.1). Next, H1N1-UI182

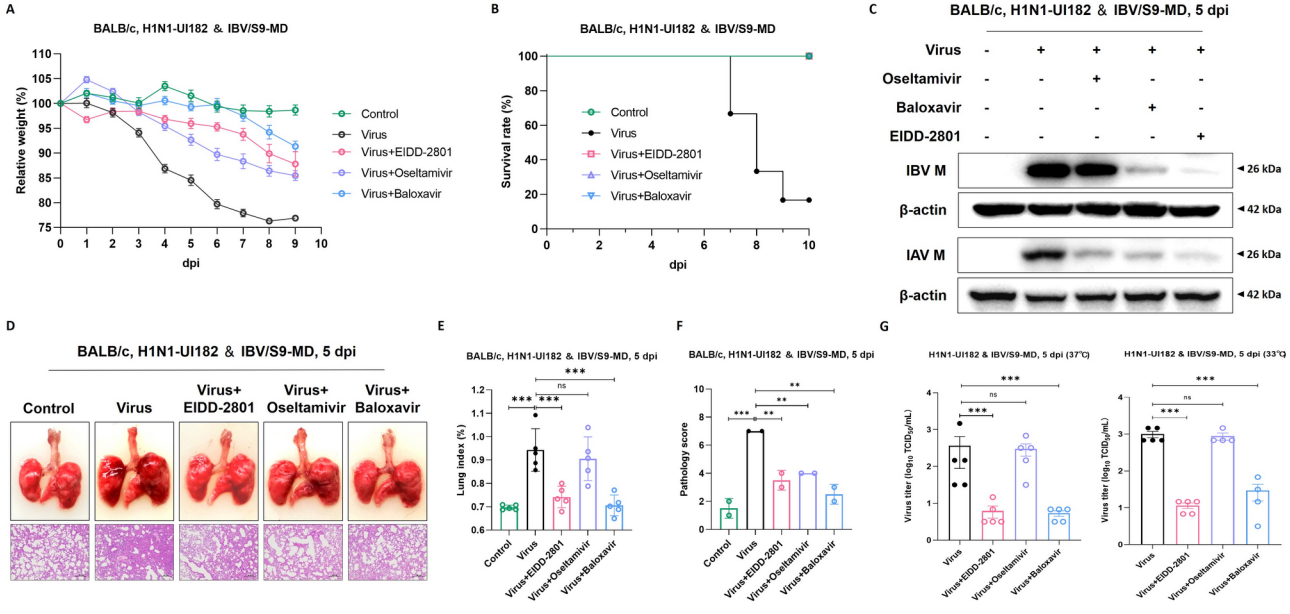

Fig. 2 EIDD-2801 protects mice from lethal co-infection of influenza A virus and influenza B virus. Mice co-infected with both H1N1-UI182 and IBV/S9-MD were monitored daily for clinical signs. **A** Body weight of infected mice. **B** Survival curves of infected mice. **C** Western Blot analysis of IAV M and IBV M protein expression in lung tissue homogenates ($n=5$). The expression of β-actin was used as loading control **D** Representative images and photomicrographs of lung tissue (H&E staining) from mice co-infected with H1N1-UI182 and IBV/S9-MD. **E** Lung index of infected mice. **F** Histopathological scores of the lung tissues. **G** Viral titers in turbinates collected from euthanized mice at 5 dpi. Differences were considered significant at (**) $0.001 < P < 0.01$, (***) $P < 0.001$, "ns" denotes no significance

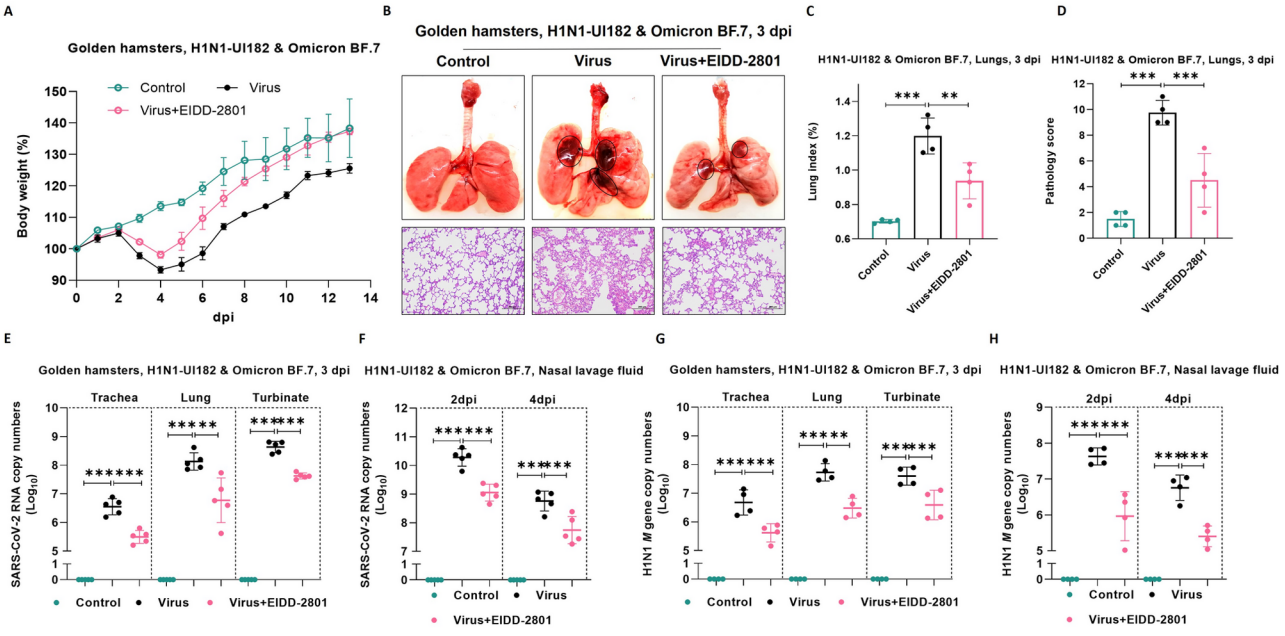

Fig. 3 EIDD-2801 improves prognosis of mice co-infected with influenza virus and SARS-CoV-2. Mice co-infected with both H1N1-UI182 and Omicron BF.7 were monitored daily for clinical signs. **A** Body weight change of co-infected golden hamsters. **B** Representative images of lung tissue are presented, with photomicrographs showing histopathological changes (scale bars = 200 μm). **C** Lung index of hamsters 3 days after infection. **D** Histopathological scores of hamster lungs. **E** Viral RNA copy numbers of SARS-CoV-2 *ORF1ab* RNA in tracheas, lungs and turbinates. **F** Viral RNA copy numbers of SARS-CoV-2 *ORF1ab* RNA in nasal lavages. **G** Viral RNA copy numbers of H1N1 *M* gene in tracheas, lungs and turbinates. **G** Viral RNA copy numbers of H1N1 *M* gene in nasal lavages. Differences were considered significant at (**) $0.001 < P < 0.01$, (***) $P < 0.001$

and Omicron BF.7 were selected to establish a co-infection model in golden hamsters. Compared with the blank control group, infected hamsters exhibited substantial body weight loss within the first 4 days post-infection, whereas EIDD-2801 treatment moderately alleviated this weight loss (Fig. 3A). Macroscopic analysis of extracted lungs revealed that extensive hemorrhage (marked by the black circles), pulmonary swelling and pathological damage caused by mixed viral infection were enormously alleviated following oral administration of EIDD-2801 (Fig. 3B), Quantitative analysis further demonstrated a marked reduction in lung index and histopathological severity score compared to virus control (Fig. 3C, D). Then we quantified viral RNA copy numbers in tracheas, lungs, turbinates and nasal lavage. qRT-PCR analysis revealed reductions in viral loads across all tissues. For SARS-CoV-2, EIDD-2801 treatment decreased *ORF1ab* RNA levels by 1 Log_{10} in tracheas, 1.5 Log_{10} in lungs, and 1 Log_{10} in turbinates compared to virus control (Fig. 3E). Notably, *ORF1ab* RNA copies in nasal lavage exhibited a reduction, with 1.3 Log_{10} decrease at 2 dpi and 1.2 Log_{10} decrease at 4 dpi (Fig. 3F). Similarly, EIDD-2801 treatment reduced H1N1 *M* gene RNA levels by 2 log_{10} in tracheas, 1.5 log_{10} in lungs, and 1 log_{10} in turbinates (Fig. 3G). Furthermore, H1N1 *M* gene RNA copies in nasal lavage exhibited a decline, with reductions of 1.6 Log_{10} at 2 dpi and 1.3 Log_{10} at 4 dpi (Fig. 3H). These results suggested that orally administered EIDD-2801 could not only inhibit viral replication of influenza A virus and SARS-CoV-2 but also markedly improve prognosis level of mixed infection of these two viruses.

Discussion

Multiple respiratory viruses, including influenza virus and coronavirus, are primarily transmitted through respiratory droplets and close contact and their co-circulation can exacerbate the severity of respiratory infections. Among these, influenza A and B viruses, which have posed significant public health challenges during the COVID-19 pandemic are highly prone to co-infect with SARS-CoV-2. Such co-infections may amplify pathogenic effects due to synergistic interactions between viral mechanisms, leading to increased disease severity [46–48]. Positive antiviral strategies including mask-wearing, social distancing, and vaccines were proven to be effective in prevention and reducing morbidity and mortality of patients [49, 50]. However, the effectiveness of vaccines may wane over time because of the high variability and immune evasion capacity of influenza viruses and SARS-CoV-2 Omicron subvariants [51, 52]. Therefore, it is essential to develop effective and broad-spectrum antiviral agents against mono-infection and co-infection of influenza viruses and SARS-CoV-2 strains. Currently, massive drug development against these viruses

has been undertaken, no fully effective agents have been approved by FDA for the treatment of co-infection of IAV and SARS-CoV-2. Take S protein-specific neutralizing antibodies (nAbs) for instance, are ineffective against influenza virus infection and were restricted in the therapeutics against newly emerging Omicron variants and subvariants [53–55].

EIDD-2801 initially emerged as a possible antiviral for alphaviruses and influenza viruses due to its conspicuous effect in cell cultures. Earlier studies showed that EIDD-2801 is efficacious against influenza A and B viruses in human airway epithelial cultures. In addition, the drug demonstrated reduced shed virus titers and inflammatory infiltrates in nasal lavages and revealed a high genetic barrier to resistance in the ferret model of influenza when administered in therapeutic dosing [29, 56]. In addition, EIDD-2801 displayed an inhibitory effect on the replication and transmission of influenza viruses in guinea pig and mouse models [30]. This ribonucleoside analog has also shown in vitro and in vivo activity against a broad range of viruses including flaviviruses, filoviruses, pneumoviruses, orthomyxoviruses, and coronaviruses by increasing the rate of mutation in the viral genome of virus to induce random low-frequency nucleoside transitions and viral error catastrophe and was in preclinical development for the treatment of influenza [30, 57, 58]. The antiviral activity against SARS-CoV-2 was quickly confirmed in precious SARS-CoV-2 clinical trials during the COVID-19 pandemic, and EIDD-2801 was authorized by EUA for treatment of COVID-19 as an oral antiviral [32, 35, 57]. EIDD-2801 retained activity against all major SARS-CoV-2 variants and can delivered orally due to its great pharmacokinetic properties compared with other approved antiviral treatments such as remdesivir and reconvalescent serum which are poorly suitable for transmission control [32, 59, 60].

In this study, we confirmed the antiviral activity of EIDD-2801 against divergent authentic influenza virus strains in vitro, as well as therapeutic in vivo effects against mono-infection and co-infection of mouse-adapted influenza A virus and B virus in BALB/c mice. EIDD-2801 is potent in inhibiting infection of SARS-CoV-2 variants and subvariants such as BA.2, BF.7, and XBB which are resistant to most nAbs and antibodies in the serum of SARS-CoV-2 patients or vaccinated people. Furthermore, the results of co-infection of IAV and SARS-CoV-2 in golden hamsters showed that EIDD-2801 can dramatically reduce viral titers in tracheas, lungs, and turbinates and alleviate lung lesions. Collectively, we have demonstrated that EIDD-2801 exhibits significant antiviral activity against divergent influenza virus and SARS-CoV-2 strains, and strong protection for lethal mono-infection or co-infection of IAV and IBV, as well as non-lethal co-infection of IAV and SARS-CoV-2. Therefore, EIDD-2801 is a promising candidate as a broad-spectrum drug for the therapeutics and prevention of single infection and co-infection of IAV and SARS-CoV-2.

Conclusions

Overall, this study demonstrates that EIDD-2801 exhibits prominent antiviral activity against influenza virus and SARS-CoV-2. These results indicate that oral administration of EIDD-2801 provides significant protection and reduces viral burden during lethal challenges involving both mono-infection and co-infection with influenza virus and SARS-CoV-2, tthereby offering valuable support for the development of broad-spectrum antiviral therapies. Currently, research on EIDD-2801's activity against other common respiratory viruses, such as respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and others, remains limited. To fully explore its potential as a broad-spectrum antiviral agent, further studies are needed to systematically evaluate its antiviral spectrum against a wider range of respiratory pathogens.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02755-1.

Supplementary Material 1

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Author contributions

Bingshuo Qian: data curation, formal analysis, methodology, software, validation, visualization, writing-original draft. Rongbo Luo: formal analysis, investigation, software, visualization. Beilei Shen: investigation, resources. Lingjun Fan: formal analysis, reviewing and editing. Junkui Zhang: reviewing and editing. Shijun Zhang: reviewing and editing. Yan Sun: reviewing and editing. Xiuwen Deng: reviewing and editing. Xiaobin Pang: project administration, writing-review and editing. Wu Zhong: project administration, writing-review and editing. Yuwei Gao: conceptualization, funding acquisition, project administration, supervision, reviewing and editing.

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This study was carried out as part of our routine work.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study and included experimental procedures were conducted in accordance with the Declaration of Helsinki, and approved by the institutional animal care and use committee of Changchun Veterinary Research Institute (IACUC-AMMS-11-2023-001). All animal housing and experiments were performed in strict accordance with ethical guidelines.

Consent for publication
Written informed consent for publication was obtained from all participants.

Competing interests
The authors declare no competing interests.

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