Molnupiravir inhibits the replication of the emerging SARS-CoV-2 variants of concern (VoCs) in a hamster infection model

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Summary:

The emergence of SARS-CoV-2 variants of concern (VoCs) has exacerbated the COVID-19 pandemic. This study shows that Molnupiravir is effective against infections with B.1.1.7 or B.1.351 variants in hamsters and therefore may have potential combating current and future emerging VoCs.

Abstract (100 words)

The emergence of SARS-CoV-2 variants of concern (VoCs) has exacerbated the COVID-19 pandemic. Currently available monoclonal antibodies and vaccines appear to have reduced efficacy against some of these VoCs. Antivirals targeting conserved proteins of SARS-CoV-2 are unlikely to be affected by mutations arising in VoCs, and should therefore be effective against emerging variants. We here investigate the efficacy of Molnupiravir, currently in phase II clinical trials, in hamsters infected with either Wuhan strain, B.1.1.7 or B.1.351 variants. Molnupiravir proved to be effective against infections with each of the variants and therefore may have potential combating current and future emerging VoCs.

Keywords

SARS-CoV-2; Antivirals; Molnupiravir; VoC, hamsters, coronavirus; B.1.351

Background

Since its emergence in Wuhan [1], China in December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide resulting in a global pandemic with more than 148 million cases and ~3.1 million deaths reported until 27 April 2021 [www.covid19.who.int]. Variants of SARS-CoV-2 variants are emerging in different parts of the world, posing a new threat of increased virus spread and potential to escape from both vaccine- and natural infection-induced immunity. So far, four major circulating SARS-CoV-2 variants of concern (VoC) have been identified; lineages B.1.1.7 (UK), B.1.351 or 501Y.V2 (South Africa), B.1.1.28.1 or P.1 (Brazil) and B.429 (California, USA) [2]. These VoC have been implicated in new massive waves of infections and new spikes in excess mortality in regions that have been heavily affected by SARS-CoV-2 [3]. Moreover, several vaccine candidates showed lower efficacy in Phase 3 clinical trials in regions of South Africa where the VoC B.1.351 is circulating [4]. Consequently, people vaccinated against SARS-CoV-2 may not all be efficiently be protected from the disease following infection with one of these new variants.

Since the emergence of new SARS-CoV-2 variants will most probably continue to happen in the future, antiviral drugs that target conserved proteins of SARS-CoV-2 could solve this issue of reduced response of variants to vaccines. Such antivirals may be expected to reduce the chance to progress to severe disease when treatment is started sufficiently early and will also have a place in a prophylactic strategy (for example in immunodeficient patients).

The ribonucleoside analogue, N4-hydroxycytidine (NHC, EIDD-1931), was initially developed as an influenza inhibitor, but exerts also broader-spectrum antiviral activity against multiple viruses belonging to different families of RNA viruses. The molecule exerts its antiviral activity via incorporation into viral RNA resulting in the accumulation of deleterious transition mutations in the nascent viral RNA, leading to error catastrophe [5] Molnupiravir (EIDD-2801, MK-4482), the orally bioavailable pro-drug counterpart of NHC [6] is effective against SARS-CoV-2 infections in Syrian hamsters [7], mice [8] and ferrets [9]. Data from a first-in-human, phase 1, randomized, double-

blind, placebo-controlled study in healthy volunteers indicate that the drug is well tolerated and that plasma exposures exceed the expected efficacious doses based on scaling from animal models [10]. The drug is currently being assessed for its potential as an antiviral treatment of SARS-CoV-2 infection in Phase 2 clinical trials of infected patients (NCT04405570, NCT04405739). Very recently interim data from a phase 2 trial with Molnupiravir demonstrated a reduction in the time required to reach negative isolation of infectious virus from nasopharyngeal swabs from participants with symptomatic SARS-CoV-2 infection [11].

We recently reported on the establishment of hamster infection models for the VoCs B1.1.7 and B.1.351. We demonstrated that no major differences in disease outcome were observed with these variants as compared to the original Wuhan strain [12]. Here, we compare the antiviral activity of Molnupiravir against different SARS-CoV-2 variants in the Syrian hamster infection model.

Methods

All virus-related work was conducted in the high-containment BSL3 facilities of the KU Leuven Rega Institute (3CAPS) under licenses AMV 30112018 SBB 219 2018 0892 and AMV 23102017 SBB 219 2017 0589 according to institutional guidelines. Briefly, 6-8 weeks female SG hamsters were treated orally with EIDD-2801 (200 mg/kg, BID) or the vehicle (i.e. the control group, BID) for four consecutive days starting one hour before intranasal infection with 50 µL containing 1×10^5 $TCID_{50}$ of SARS-CoV-2 Wuhan strain [BetaCov/Belgium/GHB-03021/2020 (EPI ISL 109 407976|2020-02-03)] [12] simply called B.1-G or B.1.1.7 (hCoV-19/Belgium/rega-12211513/2020; EPI_ISL_791333, 2020-12-21) [12] and B.1.351 (hCoV-19/Belgium/rega-1920/2021; EPI_ISL_896474, 2021-01-11) [12]. At day four post-infection (pi), the animals were euthanized for sampling of the lungs and further analysis by i.p. injection of 500 µL Dolethal (200 mg/mL sodium pentobarbital). Lungs were collected for quantification of subgenomic viral RNA using N2 primers and probes targeting the viral nucleocapsid [12], infectious virus titers and lung histopathology as described previously [12] (Fig.

1A). Housing conditions and experimental procedures were done with the approval and under the guidelines of the ethics committee of animal experimentation of KU Leuven (license P065-2020).

Results

Molnupiravir (EIDD-2801) treatment resulted in a statistically significant reduction in the viral RNA copies per mg of lung tissue with 0.7 (P=0.020), 0.9 (P=0.034) and 1.5 (P=0.016) $\log_{10}$ reduction was noted in the groups that had been infected with B.1-G, B.1.1.7 and B.1.351, respectively (Fig. 1B). Similarly, treatment significantly reduced infectious virus lung titers regardless of the SARS-CoV-2 variant used for infection (Fig. 1C). The reduction in infectious virus titers ($TCID_{50}$/mg tissue) in the lungs of hamsters infected with B.1-G, B.1.1.7 and B.1.351 was 1.8 (P<0.0001), 1.9 (P<0.0001) and 2.5 (P<0.0001) $\log_{10}$, respectively (Fig. 1C). An increase in the %weight change (on d4 compared to d0 p.i.) was observed in the molnupiravir treated-groups compared to the corresponding vehicle-treated ones, especially in case of infection with the B.1-G (P=0.020) and B.1.351 (P=0.026) variants (Fig. 1D).

In addition to viral loads, lung pathology was assessed using histopathological examination as described before [12]. Significant improvement of cumulative histopathological lung scores was also observed in all the Molnupiravir-treated groups with a reduction of median disease scores from 9.5 to 5.3 (P=0.0004), 7.8 to 5.2 (P=0.001), 8.0 to 4.3 (P=0.013) in Molnupiravir-treated hamsters infected with B.1-G, B.1.1.7 and B.1.351, respectively compared to the vehicle-treated controls for each variant (Fig. 1E, Supplementary Table S1). Hematoxylin/eosin (H&E)-stained images of lungs of lungs of the vehicle-treated hamsters infected with B.1-G, B.1.1.7 or the B.1.351 SARS-CoV-2 variants revealed extensive bronchopneumonia, perivascular oedema (black arrows) and perivascular cuff of inflammatory cells (black arrows with white heads) (Fig. 2). On the other hand, the lungs of Molnupiravir (EIDD-2801)-treated animals showed no or very focal bronchopneumonia (green triangles), no or focal perivascular inflammation (black arrows with white heads) and no perivascular oedema (Fig. 2).

Discussion

Emerging and currently circulating VoCs present new challenges to the COVID-19 pandemic. Mutations arising in these VoCs may result in variants having altered fitness in terms of virus replication and transmission, altered interactions with key host proteins, and evasion of host immune responses [2]. With extensive mutations in the spike protein, antibody resistance of VoCs B.1.1.7 and B.1.351 have been reported [13]. B.1.351, in particular, was demonstrated to be markedly resistant to multiple monoclonal antibodies generated against the N-terminal and receptor-binding domain, as well as convalescent plasma from vaccinated individuals [13]. VoCs therefore greatly threaten the efficacies of available monoclonal antibody therapies and vaccines, which have been developed to target the parent strain of SARS-CoV-2 [4,14].

In contrast, by acting at the level of viral RNA replication, Molnupiravir should be able to exert its antiviral SARS-CoV-2 activity in spite of the mutations present in the emerging VoCs. This hypothesis is confirmed here in this study, whereby Molnupiravir reduces viral RNA load and infectious virus titers in the lungs of hamsters infected with parent lineage B.1-G, and VoCs B.1.1.7, and B.1.351 all to a similar extent of about 2 to 2.5 $\log_{10}$ fold compared to non-treated, infected hamsters, with comparably significant improvements on lung pathology.

The RNA-dependent RNA polymerase (RdRp) of coronaviruses is encoded by non-structural protein 12 (nsP12) which together with the accessory proteins nsP7 and nsP8 form the core RdRp complex necessary for viral RNA replication [15]. The nsP12 consists of three main domains; the N-terminal nidovirus RdRp-associated nucleotidyltransferase domain, the interface domain and the C-terminal RdRp domain [15]. The active site of the coronavirus RdRp is formed by highly conserved residues at the C-terminal domain of nsP12 [15]. Recent cryo-EM studies for the SARs-CoV-2 RdRp in presence of active metabolites of Remdsisvir and Favipiravir revealed that both compounds were bound to the substrate-binding site of the nsP12 [15]. A proline-323-Leucine substitution in the viral nsP12 is

observed in B.1.1.7, B1.351 as well as P.1 variants [2]. This amino acid residue is located in the interface domain of nsP12 and plays an important role in the interaction with the nsP8 during replication complex formation [2]. However, none of the variants carries mutations/polymorphisms in the active site of their RdRp [2]. Furthermore, given that the residues within this active site are highly conserved, nucleosides analogues such as Molnupiravir are most likely to remain also active against new variants if they would emerge.

With the efficacy of Molnupiravir unaffected by mutations in VoCs B.1.1.7 and B.1.351, and taking into consideration that Molnupiravir showed promising initial results in a phase II clinical trial in COVID-19 patients, this compound could potentially be a pan-lineage SARS-CoV-2 antiviral agent as more VoCs emerge in the future. Recently, we reported on potent antiviral effect of the combination of Molnupiravir and Favipiravir in the SARS-CoV-2 hamster infection model [7]. By envisioning it as part of combination therapy, concerns of resistance development to Molnupiravir alone could also be greatly reduced. Consequently, Molnupiravir, and other antiviral agents targeting viral replication, may be important tools in the fight against this pandemic.

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Author Contributions

R.A. and J.N. designed the studies; R.A. and B.W. performed the studies and analyzed data; J.N. provided advice on the interpretation of data; R.A., C.S.F. and J.N. wrote the paper with input from co-authors; S.D.J provided essential reagents; P.M. isolated and initially characterized variants; R.A., C.S.F. and J.N. supervised the study; J.N. acquired funding.

Conflict of Interest Statement: None to declare.

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Figure Legends

Fig.1. Molnupiravir (EIDD-2801) reduced viral loads in Syrian hamsters infected with different SARS-CoV-2 variants. (A) Set-up of the study. (B) Viral RNA levels in the lungs of control (vehicle-treated, BID) and EIDD-2801-treated (200 mg/kg, BID) hamsters infected with 10^5 TCID50 B.1-G, B.1.1.7 or B.1.351 SARS-CoV-2 variants at day 4 post-infection (pi) are expressed as $\log_{10}$ SARS-CoV-2 RNA genome copies per mg lung tissue. Individual data and median values are presented. (C) Infectious viral loads in the lungs of control (vehicle-treated) and EIDD-2801-treated hamsters infected with the different SARS-CoV-2 variants at day 4 pi are expressed as $\log_{10}$ $TCID_{50}$ per mg lung tissue Individual data and median values are presented. D) Weight change at day 4 post-infection in percentage, normalized to the body weight at the time of infection (day 0). Bars represent means ± SD. (E) Cumulative severity score from H&E stained slides of lungs from control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters. Individual data and median values are presented and the dotted line represents the median score of untreated non-infected hamsters. All data were analyzed with the Mann–Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are from two independent experiments. The number of animals were 12 and 10 per vehicle and EIDD-2801-treated groups, respectively.

Fig.2. Molnupiravir improved histopathology of lungs of Syrian hamsters infected with different SARS-CoV-2 variants. Representative H&E images of lungs of control (vehicle-treated) and EIDD-2801-treated (200 mg/kg, BID) hamsters infected with 105 TCID50 B.1-G, B.1.1.7 or B.1.351 SARS-CoV-2 variants at day 4 post-infection (pi). Left Panel: the lungs of the vehicle-treated hamsters infected with B.1-G, B.1.1.7 or the B.1.351 SARS-CoV-2 variants showing extensive bronchopneumonia (alveoli filled with neutrophils and histiocytes), perivascular oedema (black arrows) and perivascular cuff of inflammatory cells (black arrows with white heads). Right Panel: the lungs of EIDD-2801-treated groups showed no or very focal bronchopneumonia (green triangles), no or focal perivascular inflammation (black arrows with white heads) and no perivascular oedema.*Blood vessel. Scale bar 100 µM.

Figure 1

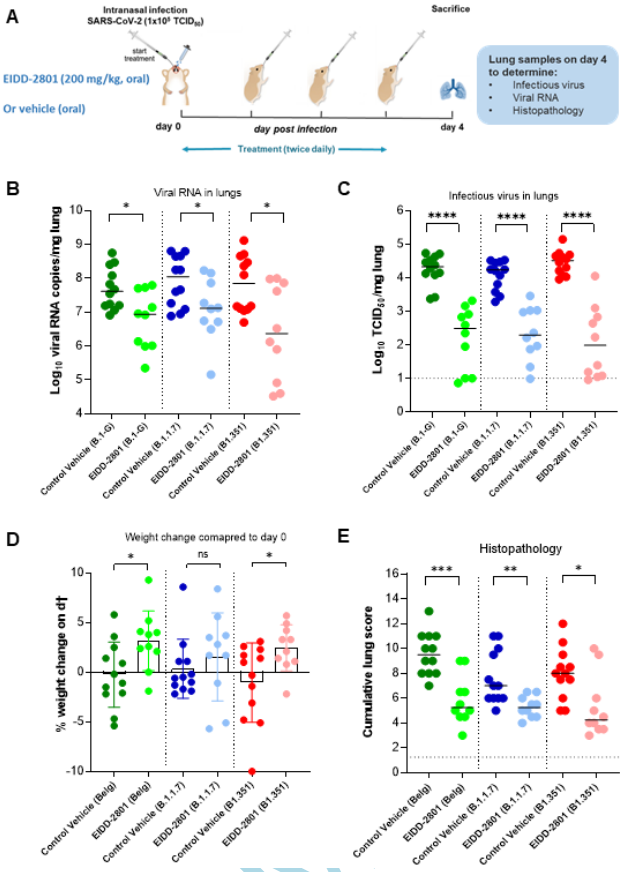

Figure 2