Pharmacokinetics of β-d-N4-Hydroxycytidine, the Parent Nucleoside of Prodrug Molnupiravir, in Nonplasma Compartments of Patients With Severe Acute Respiratory Syndrome Coronavirus 2 Infection

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β-d-N4-hydroxycytidine (NHC), the parent nucleoside of molnupiravir, a COVID-19 antiviral, was quantified at SARS-CoV-2 transmission sites in 12 patients enrolled in AGILE Candidate-Specific Trial-2. Saliva, nasal, and tear NHC concentrations were 3%, 21%, and 22% that of plasma. Saliva and nasal NHC were significantly correlated with plasma (P < .0001).

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An extended therapeutic goal of antiviral therapy is the prevention of infection in individuals who have been exposed to an infected person. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection occurs through inhalation or inoculation of virus onto upper respiratory airways and mucosal surfaces. In order to be an effective prophylactic agent, a drug must penetrate into these sites in sufficient quantities.

Molnupiravir (EIDD-2801; MK-4842), a prodrug of the ribonucleoside analogue β-d-N4-hydroxycytidine (NHC), has recently been licensed in the United Kingdom and received US Food and Drug Administration FDA (FDA) emergency use authorization (EUA) in the United States for the treatment of symptomatic coronavirus disease 2019 (COVID-19) in adults with at least 1 risk factor for developing severe disease. Following oral administration, molnupiravir is rapidly hydrolyzed by esterases to NHC, which is phosphorylated by host kinases to active intracellular metabolite EIDD-1931-5’-triphosphate (EIDD-2061) [1]. AGILE, a UK platform for early-phase trials of novel COVID-19 therapies [2], has evaluated molnupiravir within its AGILE Candidate-Specific Trial (CST)-2 seamless phase 1b/2a protocol. We previously reported phase 1b evaluation of molnupiravir across 3 dosing arms (300, 600, and 800 mg twice daily), establishing that 800 mg twice daily for 5 days was suitable for progression to phase 2 [3], which is currently recruiting.

Here, we report the pharmacokinetics of molnupiravir and NHC in saliva, nasal secretions, and tears in comparison with plasma concentrations within AGILE CST-2 1b.

METHODS

Study Design, Sampling, and Bioanalytical Methods

Molnupiravir pharmacokinetics were evaluated as part of a phase 1 dose-escalation study (300, 600, and 800 mg twice daily) in patients with polymerase chain reaction–confirmed SARS-CoV-2 infection within 5 days of symptom onset and presenting with mild or moderate disease. The study design has previously been described [3], and further details can be found in the Supplementary Materials.

Plasma and nonplasma (saliva, nasal secretions, and tears) samples were collected at pre-dose and 0.5, 1, 2, and 4 hours post-dose on study days 1 and 5. Plasma samples were collected as previously described [4]. Nonplasma sample collections and molnupiravir dosing conditions are described in the Supplementary Materials.

Molnupiravir and NHC concentrations were determined at the University of Liverpool Bioanalytical Facility (United Kingdom). Plasma and saliva concentrations were quantified using a validated liquid chromatography–mass spectrometry method [4]. NHC in nasal secretions and tears (swabs) were determined using an adaptation of this method, described in the Supplementary Materials. All assays were validated in accordance with FDA [5] and EMA guidelines [6].

Pharmacokinetic Data Analyses

Given the small sample size, representation of the pharmacokinetic data was largely exploratory and descriptive. Samples below the lower limit of quantification (LLQ; <2.5 ng/mL) at pre-dose on day 1 were included as 0 ng/mL; those less than the LLQ beyond pre-dose on day 1 were included as LLQ/2.
(1.25 ng/mL). NHC area under the concentration-time curve 0–4 hours (AUC_{0-4}), maximum concentration (C_{max}), and time to maximum concentration (T_{max}) were determined using noncompartmental analysis (Phoenix 64, WinNonlin, v. 8.3, Certara, Princeton, NJ). NHC intercompartmental nonplasma:plasma ratios (R_{NP:P}) were calculated on day 1 and day 5 for each individual using the plasma as reference (nonplasma AUC_{0-4}/plasma AUC_{0-4}). Patients without a full profile between 0 and 4 hours were excluded from AUC_{0-4} summary statistics, and those with sample(s) missing between 0 and 2 hours were excluded from C_{max} and T_{max} summary statistics.

Linear mixed-effects models were applied to evaluate the relationship between log-transformed NHC concentrations in plasma and nonplasma compartments on day 1 and day 5 (IBM SPSS Statistics v. 25.0, IBM Corporation, Armonk, NY). Concentrations below the assay LLQ were excluded.

**RESULTS**

**Patients**

Of the 12 participants (n = 4 per dosing arm), 9 (75%) were female, median (range) baseline age was 50 years (22–80), median (range) baseline weight was 79 kg (54–134), and median (range) baseline body mass index was 29 kg/m² (21–51). Time from symptom onset to randomization and start of treatment was 5 days (3–5).

Ten of the 12 individuals (83%) completed the full treatment schedule. One patient in the 300-mg cohort took 1 of 2 tablets for the second and third dose, and a participant in the 800-mg cohort withdrew after the second dose. All pharmacokinetic data were included.

**Nonplasma Samples**

Molnupiravir was detected at very low concentrations in only 31 of 106 (29%) plasma and 12 of 114 (11%) saliva samples (median [range], 5.89 [2.59–27.53 ng/mL] and 4.86 [2.63–31.44 ng/mL], respectively) and therefore not measured in swabs.

In total, 111 of 113 saliva, 112 of 112 nasal, and 96 of 106 tear concentrations were included. Sample numbers per cohort are summarized in Supplementary Table 1, and exclusions and samples less than the LLQ are outlined in the Supplementary Materials.

**NHC Nonplasma Pharmacokinetics**

NHC pharmacokinetic parameters are summarized in Table 1. Geometric mean concentration-time profiles are shown in Supplementary Figure 1; additionally, individual profiles are illustrated in Supplementary Figure 2.

### Table 1. Geometric Mean (CV%) β-d-N4-Hydroxycytidine Pharmacokinetic Parameters From Plasma, Saliva, Nasal Swabs, and Tear Strips From Severe Acute Respiratory Syndrome Coronavirus 2–Infected Patients Following Single- (Day 1) and Multiple-Dose (Day 5) Molnupiravir 300 mg, 600 mg, and 800 mg Twice Daily

| Parameter | Day 1 | Day 5 | Day 1 | Day 5 | Day 1 | Day 5 |
|-----------|-------|-------|-------|-------|-------|-------|
|           | 300 mg |       | 600 mg |       | 800 mg |       |
| Plasma    |       |       |       |       |       |       |
| AUC_{0-4} (ng h/mL) | 3031 (45) | 2328 | 5690 (22) | 4368 (41) | 8187 (30) | 7005 (21) |
| C_{max} (ng/mL) | 1488 (31) | 1048 (17) | 2440 (17) | 1865 (61) | 3447 (32) | 3546 (13) |
| T_{max} (h) | 2.00 (1.00–2.00) | 1.00 (1.00–1.00) | 1.00 (1.00–2.00) | 1.00 (1.00–2.00) | 2.00 (2.00–2.00) | 2.00 (2.00–2.00) |
| Saliva    |       |       |       |       |       |       |
| AUC_{0-4} (ng h/mL) | 65 (109) | 106 (93) | 143 (120) | 106 (77) | 289 (52) | 237 (36) |
| C_{max} (ng/mL) | 29 (113) | 41 (98) | 73 (127) | 48 (76) | 134 (48) | 109 (71) |
| T_{max} (h) | 1.00 (1.00–2.00) | 1.50 (1.00–2.00) | 2.00 (1.00–2.00) | 2.00 (2.00–4.00) | 2.00 (1.00–2.00) | 2.00 (2.00–2.00) |
| R_{NP:P} | 0.03 (62) | 0.03 | 0.04 (79) | 0.03 (51) | 0.04 (33) | 0.03 (18) |
| Nasal swabs |       |       |       |       |       |       |
| AUC_{0-4} (ng h/mL) | 1061 (38) | 673 (27) | 629 (64) | 716 (67) | 2164 (50) | 1611 (73) |
| C_{max} (ng/mL) | 805 (70) | 484 (60) | 365 (75) | 321 (65) | 1076 (43) | 738 (87) |
| T_{max} (h) | 1.00 (1.00–1.00) | 1.00 (1.00–2.00) | 1.00 (1.00–2.00) | 1.00 (1.00–4.00) | 1.50 (1.00–2.00) | 2.00 (2.00–4.00) |
| R_{NP:P} | 0.41 (73) | 0.23 | 0.17 (27) | 0.17 (112) | 0.26 (26) | 0.23 (67) |
| Tear strips |       |       |       |       |       |       |
| AUC_{0-4} (ng h/mL) | 1731 (44) | 1071 (38) | 1137 (96) | 749 (50) | 1934 (90) | 722 |
| C_{max} (ng/mL) | 908 (58) | 674 (53) | 411 (100) | 508 (84) | 985 (95) | 1267 (40) |
| T_{max} (h) | 0.50 (0.50–2.00) | 1.00 (0.50–2.00) | 2.00 (0.50–4.00) | 1.50 (1.00–2.00) | 1.00 (1.00–2.00) | 1.00 (0.50–1.00) |
| R_{NP:P} | 0.77 (36) | 0.39 | 0.20 (76) | 0.17 (47) | 0.26 (121) | 0.10 |

n = 4 per dosing arm, unless stated otherwise. T_{max} expressed as median (range).

Abbreviations: AUC_{0-4}, area under the concentration-time curve over 0 hours (pre-dose) to 4 hours post-dose; C_{max}, maximum concentration; R_{NP:P}, intercompartmental ratio of nonplasma to plasma (nonplasma AUC_{0-4}/plasma AUC_{0-4}); T_{max}, time of maximum concentration.

* n = 2.

* n = 3.
NHC saliva concentrations were approximately 3% that of plasma (median [range] R_{np,p} pooled across doses: 0.03 [0.01–0.11]; n = 16); the majority of individual ratios were between 0.01 and 0.04 (n = 12). Individual NHC R_{np,p} for nasal secretions and tears were more variable (coefficient of variation [CV]: 60%, 70%, and 92% for saliva, nasal, and tears R_{np,p} respectively) and, overall, approximately 6-fold higher than saliva R_{np,p} (median [range] R_{np,p} nasal: 0.21 [0.05–0.73]; n = 17 and tears: 0.22 [0.09–1.05]; n = 12). Geometric mean (CV%) NHC R_{np,p} stratified by molnupiravir dose and study day are described in Table 1.

NHC concentrations in saliva and nasal secretions were significantly associated with paired plasma on day 1 and day 5 (P < .0001 for all analyses), whereas statistically significant relationships were not observed for paired tear and plasma NHC concentrations (day 1, P = .068; day 5, P = .344). Time post-dose was included as a repeated effect, but addition of random effects for intercept and slope did not improve the models.

## DISCUSSION

Molnupiravir, along with nirmatrelvir/ritonavir (Paxlovid), are orally administered antivirals licensed in the United Kingdom and with FDA EUA in the United States for early treatment of mild to moderate COVID-19 in adults with at least 1 risk factor for developing severe disease. Molnupiravir is currently under phase 2 evaluation within AGILE including mild to moderate COVID-19 without risk factors and in both unvaccinated and vaccinated patients. Molnupiravir is also being investigated for prophylactic use in household contacts of symptomatic COVID-19 patients (MOVe-AHEAD). Knowledge of drug accumulation within the upper airways and mucosal secretions will inform and support future research in this area.

We observed saliva NHC concentrations that were 3% that of plasma, whereas exposure in nasal secretions and tears was higher at approximately 20% that of plasma (based on pooled AUCl_{np} ratios). Of the measured saliva, nasal, and tear samples, 6%, 50%, and 61%, respectively, were within or above the NHC concentrations (day 1, P = .068; day 5, P = .344). Time post-dose was included as a repeated effect, but addition of random effects for intercept and slope did not improve the models.

There are a number of limitations of this study. The small sample size, which is typical of early-phase studies, only allowed for a descriptive interpretation of NHC pharmacokinetics and was underpowered for statistical comparisons between matrices. We used a truncated sampling schedule between 0 and 4 hours to limit infection risk; therefore, NHC elimination over the 12-hour dosing interval could not be fully assessed in nonplasma compartments. Missing samples led to exclusions from the analysis, particularly for evaluation of R_{np,p}; contributed to data variability; and limited data interpretation. Finally, the active triphosphate metabolite, EIDD-2061, was not quantified. Despite these limitations, these data add to our understanding of NHC pharmacokinetics, principally at sites of COVID-19 infection.

To our knowledge, we are the first to describe penetration of NHC into nasal secretions and tears and, to a lesser extent, into saliva. These data support the evaluation of molnupiravir as prophylaxis for SARS-CoV-2 infection.

## Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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References

1. Painter GR, Bowen RA, Bluemling GR, et al. The prophylactic and therapeutic activity of a broadly active ribonucleoside analog in a murine model of intranasal Venezuelan equine encephalitis virus infection. Antiviral Res 2019; 171:104597.
2. Griffiths GO, FitzGerald R, Jaki T, et al. AGILE: a seamless phase 1/1a platform for the rapid evaluation of candidates for COVID-19 treatment: an update to the structured summary of a study protocol for a randomised platform trial letter. Trials 2021; 22:487.
3. Khoo SH, FitzGerald R, Fletcher T, et al. Optimal dose and safety of molnupiravir in patients with early SARS-CoV-2: a phase I, open-label, dose-escalating, randomised controlled study. J Antimicrob Chemother 2021; 76:3286–95.
4. Amara A, Penchala SD, Else L, et al. The development and validation of a novel LC–MS/MS method for the simultaneous quantification of molnupiravir and its metabolite ss-d-N4-hydroxycytidine in human plasma and saliva. J Pharm Biomed Anal 2021; 206:114356.
5. US Food and Drug Administration. Bioanalytical method validation guidance for industry. 2018. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed 2 March 2022.
6. EMEA/CHMP/EWP. Guideline on bioanalytical method validation. 2009. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed 2 March 2022.
7. Cox RM, Wolf JD, Plemper RK. Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. Nat Microbiol 2021; 6:111–8.
8. Sheehan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020; 12:eabb5883.
9. Haeckel R. Factors influencing the saliva/plasma ratio of drugs. Ann NY Acad Sci 1993; 694:128–42.
10. Merck & Co. I. Fact sheet for healthcare providers: emergency use authorization for molnupiravir. 2022. Available at: https://www.fda.gov/media/155054/download. Accessed 2 March 2022.
11. Merck Sharp & Dohme (UK) Limited. Lagevrio® (molnupiravir) 200 mg hard capsules. 2021. Available at: https://www.medicines.org.uk/emc/product/10444#companyDetails. Accessed 2 March 2022.
12. Committee for Medicinal Products for Human Use. EMA assessment report: use of molnupiravir for the treatment of COVID-19. 2022. Available at: https://www.ema.europa.eu/en/documents/referral/lagevrio-also-known-molnupiravir-mk-4482-covid-19-article-53-procedure-assessment-report_en.pdf. Accessed 2 March 2022.