Pharmacokinetics of ß-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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ß-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-4482), 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/m^2 (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	300 mg		600 mg		800 mg	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
Plasma						
AUC_{0-4} (ng h/mL)	3031 (45)[a]	2328[b]	5690 (22)[c]	4368 (41)	8187 (30)	7005 (21)[c]
C_{max} (ng/mL)	1488 (31)[c]	1048 (17)[a]	2440 (17)	1865 (61)	3447 (32)	3546 (13)[c]
T_{max} (h)	2.00 (1.00–2.00)[c]	1.00 (1.00–1.00)[a]	1.00 (1.00–2.00)	1.00 (1.00–2.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)[a]
Saliva						
AUC_{0-4} (ng h/mL)	65 (109)[c]	106 (93)[c]	143 (120)	106 (77)[c]	289 (52)	237 (36)[c]
C_{max} (ng/mL)	29 (113)[c]	41 (98)	73 (127)	48 (76)[c]	134 (48)	109 (27)[c]
T_{max} (h)	1.00 (1.00–2.00)[c]	1.50 (1.00–2.00)	2.00 (1.00–2.00)	2.00 (2.00–4.00)[c]	2.00 (1.00–2.00)	2.00 (2.00–2.00)[c]
$R_{NP:P}$	0.03 (62)[a]	0.03[b]	0.04 (79)[c]	0.03 (51)[c]	0.04 (33)	0.03 (18)[c]
Nasal swabs						
AUC_{0-4} (ng h/mL)	1061 (38)[c]	673 (27)[c]	629 (64)	716 (67)	2164 (50)	1611 (73)[c]
C_{max} (ng/mL)	805 (70)[c]	484 (60)	365 (75)	321 (65)	1076 (43)	738 (87)[c]
T_{max} (h)	1.00 (1.00–1.00)[c]	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)[c]
$R_{NP:P}$	0.41 (73)[a]	0.23[b]	0.17 (27)[c]	0.17 (112)	0.26 (26)	0.23 (67)[c]
Tear strips						
AUC_{0-4} (ng h/mL)	1731 (44)[c]	1071 (38)[c]	1137 (96)[c]	749 (50)[a]	1934 (90)[c]	722[b]
C_{max} (ng/mL)	908 (58)[c]	674 (53)[c]	411 (100)[c]	508 (84)	985 (95)[c]	1267 (40)[c]
T_{max} (h)	0.50 (0.50–2.00)[c]	1.00 (0.50–2.00)[c]	2.00 (0.50–4.00)[c]	1.50 (1.00–2.00)[a]	1.00 (1.00–1.00)[c]	1.00 (0.50–1.00)[c]
$R_{NP:P}$	0.77 (36)[a]	0.39[b]	0.20 (76)[c]	0.17 (47)[a]	0.26 (121)[c]	0.10[b]

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.

[a] n = 2.

[b] n = 1.

[c] 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 postdose 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 AUC_{0-4} ratios). Of the measured saliva, nasal, and tear samples, 6%, 50%, and 61%, respectively, were within or above the NHC 90% effective concentration (EC_{90}) against SARS-CoV-2 in primary human airway epithelia cultures [7, 8] (approximately 0.5–1 µM ≈ 130–260 ng/mL), suggesting that therapeutic concentrations are potentially attained within the nasal and ocular compartments but not in saliva. However, it is important to note that without established pharmacokinetic/pharmacodynamic relationships or virological data, further studies are warranted to determine whether efficacious or prophylactic targets are obtained in nonplasma compartments.

NHC appeared to exhibit similar absorption and elimination profiles in the matrices studied, confirmed by statistically significant linear relationships between plasma NHC with that in nonplasma compartments (with the exception of tears). A strong correlation between saliva and plasma NHC

concentrations implies (assuming a 1-compartment model) that salivary accumulation is dependent on the plasma concentration. Mucosal permeability and protein binding are major factors in determining salivary drug accumulation, since only unbound drug is available for diffusion into saliva [9]. NHC exists predominantly in unbound form in plasma (unbound fraction ≥0.99), and in vitro studies demonstrated that molnupiravir and NHC are not substrates for major drug transporters (eg, ABCB1, p-glycoprotein) [10, 11]. However, NHC is a substrate for human nucleoside transporters in vitro (eg, CNT1, ENT2) [12], which could modulate nonplasma concentrations of NHC in addition to other factors relating to the drug characteristics or surrounding milieu. Passage of drugs into nonplasma compartments can also be attributed to factors such as pH (eg, mouth), inflammation (eg, eye), and flow rate. For example, pharmacokinetics of drug in tears may be affected due to increased lacrimation or infection. Higher turnover or flow rate of saliva may also contribute to the lower concentrations observed. Additionally, the marked variability in nasal and tear NHC concentrations could be associated with the challenging collection procedures.

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

Notes

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