In Vitro Assessment of Antiviral Chemotherapy Against Human Coronaviruses

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ABSTRACT

Three antiviral drug compounds are licensed in the United States for the prevention and therapy of SARS-CoV-2 and its variants. These drugs are remdesivir, paxlovid, and molnupiravir. These drugs are only prescribed for people who are hospitalized with COVID-19 and need supplemental oxygen or have a higher risk of serious illness. Other drug compounds have been approved for prevention and therapy of SARS-CoV-2 and its variants but are only authorized for emergency use only. Molnupiravir is used to treat mild to moderate COVID-19 in adults who are at higher risk of serious illness (1).

This study will evaluate the Delta and Omicron variants of the SARS-CoV-2 virus against the antiviral drug molnupiravir. These viruses will be tested in drug sensitivity assays, and EC_{50} values will be determined for each drug by visual observation and neutral red staining in three replicate experiments.

These studies will demonstrate whether the Delta and Omicron variant isolates of SARS-CoV-2 remain sensitive to different doses of molnupiravir in vitro and if combination chemotherapy holds promise as a possible treatment strategy for drug-resistant SARS-CoV-2 and its variants.

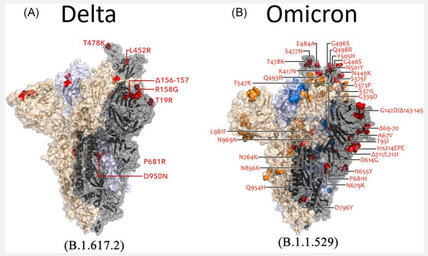

Figure 1: A model of the Spike proteins from the Delta and Omicron variants.

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INTRODUCTION

Understanding antiviral drug effects on drug-resistant viruses allows for proactive treatment options for new and emerging viruses. It is difficult to create effective antivirals for SARS-CoV-2 and other coronaviruses. This is an issue of great concern. Combination chemotherapy is one possible strategy for the treatment of drug-resistant virus infections. Strains of the ancestral SARS-CoV-2, SARS-CoV, and MERS-CoV viruses were obtained from Dr. Guy Boivin (Laval University, Quebec). These virus strains have been previously characterized in vitro and shown to differ in their sensitivities to remdesivir, interferon ***β***-1a, and other compounds (2).

METHODS

Virus stocks of the Delta and Omicron variants of SARS-CoV-2 were prepared in the Institute for Antiviral Research. Antiviral drugs for this study will include molnupiravir and will be provided by the Institute of Antiviral Research. All antiviral tests will be completed in triplicate and used equal virus titers.

Virus yield reduction (VYR) assays will be completed in 96-well microplates infected with approximately 50 cell culture infectious doses ($CCID_{50}$) of virus. Virus yields will be determined from samples collected on day 3 of the infection when untreated control wells exhibited 100% cytopathic effects (CPE). Two microwells will be used per dilution of inhibitor. Virus will then be quantified by end-point dilution in 96-well plates of Madin-Darby Canine Kidney (MDCK) cells and virus titers calculated by the method of Reed and Muench (2,3). Four microwells will be used per dilution. Plates will then be visually examined for appearance of virus-induced CPE and neutral red will confirm cell destruction.

Drug combination effects will be determined using the three-dimensional analysis program, MacSynergy™ II, of Prichard and Shipman (3). Synergy in VYR data will then be defined as a one log (or greater) decrease in virus titer. Antagonism will be defined as a one log (or greater) increase in virus titer. Indifferent will be defined as no clear synergy or antagonism.

SARS-CoV (Vero 76 cells)

Compound	CC_{50}	EC_{50}	SI_{50}	EC_{90}	SI_{90}
Lopinavir	21.9 ± 12.8	>17.0 ± 1.0	0	12.2 ± 0.2	1.4
Chloroquine	25.3 ± 13.0	1.3 ± 0.2	18	4.0 ± 0.4	6.3
Remdesivir	91.6 ± 11.5	1.2 ± 0.7	94	1.1 ± 0.5	103
Interferon ***β***-1a	>10 ± 0.0	<0.0013 ± 0.002	> 3200	<0.0013 ± 0.002	3162
Favipiravir	>100.0 ± 0.0	>100.0 ± 0.0	0	>67.7 ±55.9	11

MERS-CoV (Vero 76 cells)

Compound	CC_{50}	EC_{50}	SI_{50}	EC_{90}	SI_{90}
Lopinavir	21.9 ± 12.8	10.3 ± 6.7	1.4	7.8 ± 4.9	2.9
Chloroquine	25.3 ± 13.0	4.8 ± 1.5	3.2	4.1 ± 0.5	4.7
Remdesivir	91.6 ± 11.5	2.4 ± 0.3	> 32	1.8 ± 0.6	>57
Interferon ***β***-1a	>10 ± 0.0	<0.00032 ± 0.0	>3200	<0.00032 ± 0.0	>3125
Favipiravir	>100.0 ± 0.0	>69.5 ± 12.7	0.3	58.1 ± 36.3	>2.1

SARS-CoV-2 (Vero 76 cells)

Compound	CC_{50}	EC_{50}	SI_{50}	EC_{90}	SI_{90}
Lopinavir	21.9 ± 12.8	>30.3 ± 23.7	0	18.4 ± 14.8	1.9
Chloroquine	25.3 ± 13.0	13.7 ± 9.7	3	6.0 ± 7.7	16
Remdesivir	91.6 ± 11.5	8.3 ± 9.3	27	5.1 ± 5.5	39
Interferon ***β***-1a	>10 ± 0.0	<0.0012 ± 0.002	>3200	<0.001 ± 0.002	>3149
Favipiravir	>100.0 ± 0.0	70.0 ± 38.4	1.5	43.7 ± 46.4	>9.7

Table 1: Drug interaction against SARS-CoV-2 and similar viruses in MDCK cells.

RESULTS

The effective concentrations (EC_{50}) of molnupiravir will be calculated for both the Delta and Omicron variants of SARS-CoV-2 and will be shown in a table below. Such calculations will include the mean and standard deviation of the independent assays in MDCK cells. The EC_{50} is the drug concentration required to inhibit virus growth by 50%. This data demonstrate will the drug sensitivity or resistance of the Delta and Omicron variants of SARS-CoV-2 to molnupiravir.
As this study has not yet commenced, there are no results for the effectiveness of molnupiravir against the Delta and Omicron variants of SARS-CoV-2. Preparation for this study is underway, including learning effective care and breeding of MDCK cells as well as learning the technique of how to transfer media and viruses in a sterile manner.

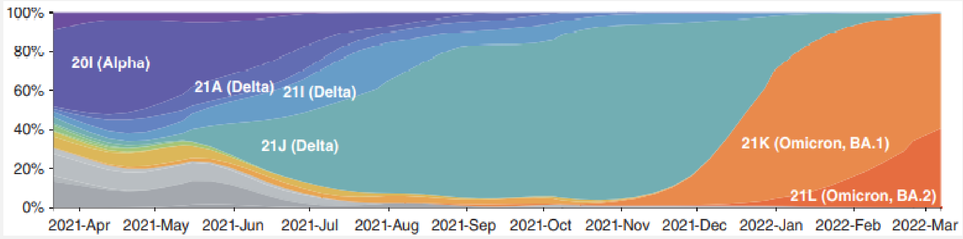

Figure 2: A model showing the concentration of SARS-CoV-2 variants from April 2021 to March 2022 (4)

CONCLUSIONS

These studies will either confirm or deny the antiviral drug resistance profile of variants of SARS-CoV-2 and determined the ability of molnupiravir chemotherapy to inhibit drug-resistant virus from replicating in MDCK cells.

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