Study of *in vitro* Remdesivir Resistance for Severe Acute Respiratory Syndrome Coronavirus 2 (COVID-19)

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Introduction

Coronaviruses are a family of enveloped, single stranded RNA viruses that cause respiratory and intestinal infection in both humans and animals. There have been many identified coronaviruses that cause infection to humans, such and HCov-229E and HCoV-OC43 which cause the common cold. Middle Eastern Respiratory virus (MERS) and Severe Acute Respiratory virus (SARS) are other notable coronaviruses that have had high mortality outbreaks. The most infamous coronavirus to date which is responsible for the current pandemic the entire world faces is known as COVID-19, or SARS2 for its similarity to SARS. As the pandemic carries on, Remdesivir has been a drug widely looked to as a potential medicine for the novel coronavirus and was even administered to Trump. With all drug treatments, there is the worry for development of resistant strains of the virus. My study attempts to develop a remdesivir-resistant strain of SARS2. This study will give us insight into if remdesivir resistance can be achieved. Further studies testing the fitness of the resistant strain can be performed and help prepare us to fight against a remdesivir resistance coronavirus strain that could arise naturally.

Objective

The goal of my research project is to develop a strain of Severe Acute Respiratory Syndrome Coronavirus 2 (COVID-19) that is resistant to the antiviral drug Remdesivir. Antiviral resistance can occur through new mutations of the virus and can be shown through EC50 values.

- EC50: The effective concentration of the drug that produces a response halfway between the baseline and the maximum response.
- The baseline is the cell control, in which no drug or virus had been added.
- The maximum response is the virus control, in which only virus has been added to the cells with no drug.

Therefore, the EC50 is the value halfway between the cell controls and virus control. I am hoping to see an increase in the EC50 value of my virus strain, meaning that a higher concentration of drug much be added to produce the same effect.

Methods

1.Drug Prep

96-well cell culture plates containing Vero 76 cells seeded at 3e4 were prepared 24hrs in advance. Half-log dilutions of Remdesivir were performed in media such that the final concentrations on the plate ranged from 0.032-100uM. Drug was added to the first five columns of the 96-well plate. Column 6 and 7 contained the cell control wells, blanks, and virus control wells, and the remaining 5 columns 8-12 did not contain any drug, only media. Layout of the plates can be visualized in Figure 1.

2.Infection

Virus stock of SARS-CoV-2 of the USA_WA1/2020 strain was diluted 1:1000 in test media and added to the test wells and virus control wells. No virus was added to columns 1 and 2. These wells are used to determine toxicity of the drug. Virus was grown at 37°C until virus control wells contained 80-100% cytopathic effect, or CPE, usually around Day 3 to 5.

3.Collection/Reading

CPE of each well was assessed visually, and media from test wells containing partial CPE were collected. This media was used as the next passage of the virus. CPE of the plates were also assessed via Neutral Red staining. Neutral Red stain will be taken in by alive cells. Therefore, unaffected healthy cells will stain darker than cells killed by virus. Plates were read with a plate reader and the average Neutral Red values for the cell controls and the virus controls were taken. These values were used to calculate the EC50 for Remdesivir.

4.Growth of New Virus Passage

Media collected from test wells containing partial CPE were added to T-25 flasks containing Vero 76 cells at about 80% confluency. Virus was grown until 40-60% CPE was achieved, usually around day 3. The new virus stock underwent one freeze/thaw cycle, and then was centrifuged to remove cell debris. The new stock was then aliquoted into cryovials and used to infect the next plate containing Remdesivir.

uM	Tox	Virus	Cell Control	Virus	Tox	uM
100						100
32						32
10						10
3.2						3.2
1.00						1.00
0.32						0.32
0.10						0.10
0.032						0.032
	Tox	Virus	Virus Control	Virus	Tox	

Figure 1. Map of 96-well plate. Red shading is used to distinguish Neutral Red staining

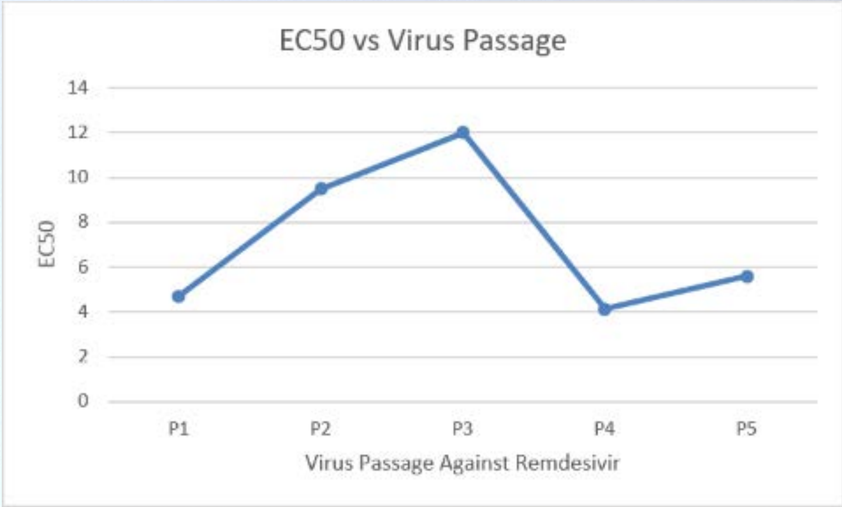

Figure 2. Graph of EC50 for each virus passage

Results

The first three passages of the virus were promising. The EC50 increased from 4.7 to 9.5 to 12. In other words, more drug was needed to produce a 50% effective concentration. However, after the fourth passage the EC50 dropped to 4.15. This was discouraging as is showed the following passage to be LESS resistant to Remdesivir than the previous passage. After the fifth passage the EC50 climbed slightly to 5.6

Conclusion

Overall, I did not achieve my goal of developing a Remdesivir resistant stock of Severe Acute Respiratory Coronavirus 2. The EC50 of my first three passages climbed steadily but on the fourth passage the EC50 dropped down to below the original stock. The EC50 did not rise significantly in the fifth passage. I am planning on continuing my project on to next semester. I may try starting over from passage three and see if the EC50 will rise. If I see no significant increase in EC50 after passage seven or eight I will try different methods to produce a remdesivir resistant virus.

References

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