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 recently been a drug considerered as a potential medicine for the novel coronavirus. As with majority of 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 must be added to produce the same effect.

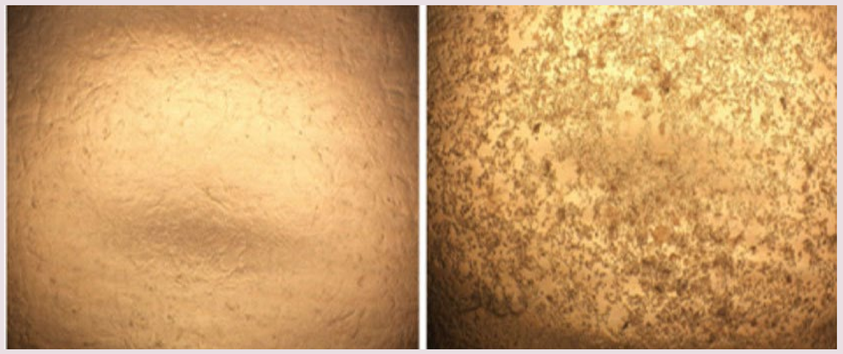

Figure 1. The picture on the left is an example of healthy cells that have not been effected by virus. The right picture is an example of the cytopathic effect produced by viral infection (Algaissi and Anwar, 2020) Healthy cells will take up stain while dead cell (right) will not.

uM	Tox		Virus			Cell Control		Virus			Tox	
100	1.65	1.855	1.54	1.5	1.485	1.503	1.613	0.144	0.143	0.128	1.979	1.609
32	1.691	1.696	1.801	1.595	1.904	1.797	1.604	0.142	0.122	0.126	1.876	2.152
10	1.603	1.659	0.337	0.232	0.305	1.45	1.544	0.143	0.128	0.133	1.624	1.711
3.2	1.317	1.565	0.205	0.172	0.16	0.054	0.053	0.145	0.135	0.126	1.76	1.592
1.00	1.882	1.653	0.116	0.163	0.138	0.053	0.051	0.157	0.133	0.136	1.519	1.851
0.32	1.256	1.623	0.155	0.15	0.142	0.138	0.145	0.13	0.149	0.13	1.499	1.774
0.10	1.677	1.527	0.131	0.152	0.162	0.164	0.141	0.127	0.132	0.127	1.577	1.509
0.032	1.302	1.498	0.141	0.147	0.163	0.155	0.13	0.154	0.145	0.144	1.569	1.949
	Tox		Virus			Virus Control		Virus			Tox	

Figure 2. Neutral Red absorbance values from passage 9 of virus with remdesivir.

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.

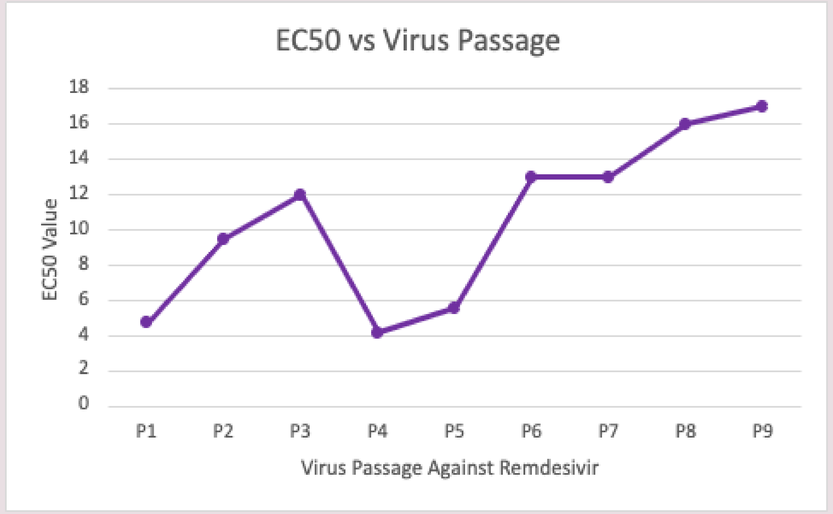

Figure 2. Graph of EC50 for each virus passage

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. The following passages saw an increase in resistance to an EC50 of 17 in the 9th passage.

Conclusion

Overall, I did not achieve my goal of developing a Remdesivir resistant stock of Severe Acute Respiratory Coronavirus 2. Although the EC50 values increase from 4.7 up to 17, this is not a significant enough difference for the virus stock to be considered resistant to remdesivir. Ideally, the EC50 value should increase with each new passage of the virus with remdesivir, and the EC50 value of the final passage should be many-fold higher that the original EC50 value. In my experiment, the EC50 value fluctuated from increasing to decreasing, and the final value was not significantly higher than the original value.

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