ENHANCEMENT OF FAVIPIRAVIR WITH COMPOUND X IN A HAMSTER MODEL OF YELLOW FEVER VIRUS INFECTION

by

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Capstone submitted in partial fulfillment of the requirements for graduation with

University Honors

with a major in
Human Biology

in the Department of Biology

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Spring 2024

Abstract

Yellow fever virus (YFV) has long been a worldwide health concern, and recent outbreaks in South America and Africa have resulted in significant mortality. There is currently an effective vaccine to prevent infection, but there are no currently developed antiviral drugs to treat patients that have already contracted the disease. Because of this, an effective antiviral is needed to combat unanticipated cases or emergence in areas that have previously been unaffected by this virus. A drug called favipiravir has shown promising results previously. This compound acts as a nucleoside analog to halt the replication of the virus and stop infection. However, the needed dosages of favipiravir are toxic in humans. A separate drug termed "Compound X" has shown potentiator activity by increasing the concentration of the active form of favipiravir in vitro. In this study, we administered suboptimal, non-toxic doses of favipiravir in combination with Compound X to determine this combination treatment's antiviral efficacy against YFV infection in an in-vivo, hamster model. Different doses of favipiravir alone, Compound X alone, a vehicle placebo drug, or both favipiravir and Compound X in tandem were administered twice daily for seven days. Mortality and weight change were recorded for the course of 21 days. Serum was collected on days four and six post infection and analyzed for serum virus titers as well as alanine aminotransferase (ALT) levels indicative of YFV infection. No significant difference was seen in serum viremia or ALT levels in any groups. However, there was a significant difference in weight change and mortality among the groups treated with the combination treatment. The combination treatment of Compound X with favipiravir was more effective than either drug alone at reducing weight loss and overall mortality in the groups treated. These results show promise for the future that these drugs may be used in combination to effectively treat YFV infection while avoiding toxicity in humans.

Acknowledgements

This project has been a collaboration from the beginning, and I am grateful for the efforts of so many people that assisted me in its completion. First, I would like to thank my faculty mentor, Dr Justin Julander for his support in me undertaking a project of my own, as well as his guidance in learning how the field of antiviral research functions. Thank you for your encouragement and passion in answering the tough questions I would always come to you with.

I'd also like to thank my departmental honors advisor, Dr Sara Freeman, for teaching me how to be a researcher in the first place and for providing me feedback throughout the duration of this study.

Thanks to all my fellow research technicians and coworkers at the Utah State University Institute for Antiviral Research in assisting me in the brunt work of dosing, weighing, infection, and collecting serum for analysis, I could not have done this without you. Special thanks to my family, friends, and wife who supported me, gave me advice, and helped me throughout the entire process.

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Introduction

Yellow fever virus (YFV) has long been a worldwide health concern. Since the 18th century, there have been recurrent outbreaks continuing until today, with 30,000-60,000 deaths yearly from this disease (Douam and Ploss, 2018). This virus is transmitted from infected monkeys to mosquitoes, who then infect humans when bitten. This mode of transmission makes the virus impossible to eradicate entirely (Monath 2001). Recent outbreaks due to vaccine shortages in Angola in 2016 and Brazil in 2018 have raised the alarm again for this life-threatening disease (Douam and Ploss, 2018). Though there is an effective preventative vaccine developed, there is no antiviral compound currently approved for use with humans to treat those who have already contracted YFV (Patel and Simons, 2013).

There have been many compounds tested against YFV infection in the past, with varying efficacy. An antiviral compound called favipiravir has shown promise in the past for treating YFV. This drug acts as a nucleoside analog; inside the cell, it is converted into its active form, ribosylated favipiravir triphosphate, and inserted into the viral genome. When inserted, it halts viral replication and infection altogether. Though this drug is effective at treating the disease, the needed doses of favipiravir have shown to be toxic in humans. Combination treatments have seen the most efficacy, in which two drugs are used simultaneously (Julander et. al., 2006). A potentiator drug termed "Compound X" has shown promise *in vitro* by increasing the concentration of ribosylated triphosphate favipiravir inside the cell by either assisting in its conversion or inhibiting its breakdown back to its active form. Since this combined treatment has shown promise in cell culture, the current study aimed to test the efficacy of this combination treatment in an *in vivo*, hamster model. The Syrian Golden Hamster has been identified as a viable model organism in YFV infection due to its similar pathology in humans (Julander, 2016).

The purpose of this study was to verify the efficacy of Compound X in combination with favipiravir in treating YFV infection. We hypothesized that administering suboptimal, non-toxic doses of favipiravir, in tandem with varying doses of Compound X, would effectively treat YFV infection while simultaneously avoiding toxicity. As we improve our understanding of the relationship between these two, we may better develop compounds in the future that can alleviate the suffering of those already infected with YFV and prevent mortality from outbreaks that will inevitably arise.

Methods

This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University (Protocol #10010). The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University.

This study was divided into two separate portions: a toxicity study and a viral challenge study. The purpose of the initial toxicity study was to verify whether our dosages of favipiravir and Compound X caused any weight loss or toxicity signs in non-infected hamsters. For this portion of the study, 18 female (female hamsters typically show more virulence with YFV infection) Syrian golden LVG/Lak strain hamsters were ordered from Charles River Laboratories. These animals were block randomized by weight, individually marked with ear tags, separated into 6 groups, and quarantined for three days upon arriving at the facility. Protocol at the Utah State University Institute for Antiviral Research is to number non-infected groups with even numbers and infected groups with odd. Therefore, for the toxicity portion of this study, groups were numbered 2, 4, 6, 8, 10, and 12 respectively. Different dosages of the two

drugs were then prepared (Table 1). The treatment was then administered to all subjects twice daily for one week, according to Table 1. Favipiravir was administered orally twice a day, and Compound X was administered intraperitoneally once a day. Compound X was dissolved in a vehicle solution of WFI (water for injection). Favipiravir was dissolved in a bicarbonate buffer solution. As a control, group 12 received only the vehicle solution, both intraperitoneally once a day and orally twice a day. Animals were weighed daily for weight, and monitored for mortality, and toxicity signs daily. Toxicity signs included hunching, lethargy, and ruffled coats. Following the first week, the treatment was terminated, and hamsters were monitored for weight change every other day and mortality/toxicity signs daily for seven more days, at which time the initial toxicity portion of the study was terminated.

Upon verifying that the required dosages were non-toxic, 95 female Golden Syrian LVG/Lak strain hamsters were ordered from Charles River Laboratories. These animals were block randomized by weight, individually marked with ear tags, separated into eleven different groups, and quarantined for three days upon arriving at the facility. Infected groups were numbered 1, 3, 5, 7, 9, and 11, and non-infected groups were numbered 2, 4, and 6. A virus challenge dose of 200 $CCID_{50}$ (cell culture infectious dose 50%) per hamster (approximately 6 times the lethal dose in hamsters) was then administered via bilateral intraperitoneal injection of 0.1 ml. Jimenez hamster-adapted yellow fever virus strain (V#2653) was used as the challenge. Groups 2, 4, and 6 were used as controls and were not infected. The 11 different groups were then given differing doses of favipiravir alone, Compound X alone, the two drugs combined, or a vehicle twice daily, according to Table 2. Favipiravir was given twice daily orally, and Compound X was given once daily intraperitoneally. Control groups received the vehicle alone for each drug. Treatments were initiated 8 hours prior to virus challenge for group 13, 4 hours

prior to virus challenge for groups 9 and 11, and 8 hours following virus challenge for the remaining groups. Weight change was monitored every other day, and mortality was monitored twice daily for each individual hamster throughout the week-long treatment. Following the first seven days, treatment with the two drugs and vehicle was terminated, and hamsters were simply monitored for weight every other day and mortality every other day for two more weeks.

On the fourth day post-infection (dpi), blood was collected via ocular sinus bleed then spun in heparinized tubes at 10,000 rpm for 5 minutes to collect serum from all hamsters, then frozen at -80° C. Serum was then used to analyze virus titers using an infectious cell culture assay where a specific volume of serum was added to the first tube of a series of dilution tubes. 50:450 dilutions were then made and added to Vero 76 cells. These cells are derived from non-human primate kidneys and have been shown to effectively model YFV *in vitro*. Ten days later, a technique measuring cell death called cytopathic effect (CPE) was used to identify the end-point of infection. Four replicates were used to calculate the CCID50 per mL of plasma or gram of tissues.

On dpi 6, blood was again collected via ocular sinus bleed then spun in heparinized tubes at 10,000 rpm for 5 minutes. Serum was then used to test for alanine aminotransferase (ALT) levels in the bloodstream of each individual hamster. ALT is an enzyme released from the liver when it is damaged. YFV typically targets the liver, leading to liver damage and significantly increased ALT levels with infection. ALT reagent (Teco Diagnostics, Anaheim, CA) was used, and the existing protocol was adapted for use in 96-well plates. Briefly, 50 µl aminotransferase substrate was placed in each well of a 96-well plate, and 15 µl of sample was added at timed intervals. The samples were incubated at 37˚C, after which 50 µl color reagent was added to each sample and incubated for 10 min. A volume of 200 µl of color developer was next added to each

well and incubated for 5 min. The plate was then read on a spectrophotometer, and ALT concentrations were determined per manufacturer's instructions.

Survival data were analyzed using the Wilcoxon log-rank survival analysis. All other statistical analyses were done using one-way ANOVA using a Dunnett multiple comparison (Prism 5, GraphPad Software, Inc).

Results

The drugs favipiravir and Compound X were given in different combinations and dosages (Table 1) to 18 uninfected, healthy adult female hamsters across 6 groups to monitor for potential toxicity effects across a two-week period. We saw consistent weight gain across all 18 hamsters for the full two weeks, and no toxicity signs were observed, allowing us to proceed with the viral challenge portion of the study.

The potentiator Compound X was tested for therapeutic activity in combination with a suboptimal dose of Favipiravir to prevent viral infection in a hamster model of YFV. The treatments with 200 mg/kg/d favipiravir+40 mg/kg/d Compound X, 200 mg/kg/d favipiravir+20 mg/kg/d Compound X, and 100 mg/kg/d favipiravir+20 mg/kg/d Compound X resulted in significant improvement in survival of YFV-infected hamsters as compared with placebo (Figure 1, Table 3). Interestingly, despite this initial improvement, there was one mortality late in the study (day 13) in the 200+40 treated group. Treatment with either 200 or 100 mg/kg/d of favipiravir in combination with 20 mg/kg/d of Compound X 8 hours post inoculation were most effective in decreasing mortality (Figure 1, Table 3). 200 mg/kg/d of favipiravir was used as a positive control but did not result in significantly increased survival as compared to placebo. A

40% mortality rate was observed after challenge of untreated hamsters infected with YFV (Figure 1, Table 1), which is much lower than the 60% mortality that is typically measured after untreated infection with YFV in hamsters (Julander, 2016). This lower-than-expected mortality and unexpected ineffectiveness of the positive control could be due to a variety of factors. The viral dilution could have been allowed to return to room temperature for too long, making the infection less effective, which would explain the later mortality date trend (Figure 1), as compared to the usual high mortality in days 4-7. Technician error could have also played a role in ineffective dosing and/or infection. However, overall, it is clear that combination treatment of favipiravir with Compound X resulted in significant improvement in mortality for infected hamsters treated 8 hours post inoculation compared to favipiravir alone.

A similar upward trend in weight change was seen across the following groups: the controls, 200 mg/kg/d favipiravir treated groups, treatment with 200 mg/kg/d favipiravir+20 mg/kg/d Compound X, and sham infected groups treated with vehicle (Figure 2). The group treated with only vehicle followed a similar trend until about day 13 post infection, where they saw a leveling out and slight decrease in mean weight change. This unexpected, consistent increase in mean weight for the vehicle-treated group could be due to the aforementioned errors with infection. The treatments for 200+40 (with a p value<0.05) and 100+20 saw a mean increase in percent weight change at a slightly slower rate than the aforementioned groups, and the Compound X-only treated group saw slower growth still (Figure 2). The group treated with 100mg/kg/d of favipiravir saw a sharp decrease in mean percent weight change at day 10, indicating this dosage and treatment alone was ineffective at treating YFV infection. Altogether, these results indicate that combination treatments of favipiravir and Compound X are more effective at alleviating infection-induced weight loss than either alone. Sham-infected hamsters

treated with placebo and uninfected, untreated normal control hamsters had an overall consistent increase in weight over the course of the study.

Serum was collected 4 dpi in order to analyze virus titers. Viremia measures the amount of virus found in the blood of an infected animal and is a reliable measure for sickness. None of the groups had significantly different viremia titers as compared with the infected vehicle treatment group (Figure 4, Table 3). Ineffective infection could have led to decreased viremia titers at 4 dpi when serum was collected. Alanine aminotransferase levels showed similar results to viremia titers, with no significant difference between any of the groups (Figure 5, Table 3).

Discussion

This study had mixed results, some of which show promise for future research. The toxicity study verified that the dosages of favipiravir and Compound X we administered for the challenge portion of the study had no toxic effects on hamsters across an extended period of time. This allows us to rule out drug toxicity as a cause for any weight loss or mortality seen in a challenge study.

The specific treatments of 200 mg/kg/d favipiravir +40 mg/kg/d Compound X, 200 mg/kg/d favipiravir+20 mg/kg/d Compound X, and 100 mg/kg/d favipiravir+20 mg/kg/day of Compound X all showed significant differences in survival as compared to control groups (Figure 1). This result indicates that the combination treatment of favipiravir with Compound X was more effective than vehicle alone at treating YFV in an in-vivo model. Additionally, because we saw no significant difference in mortality between the favipiravir-only or Compound X-only treated groups and placebo groups, we can logically conclude that the combination treatment is

also more effective than either treatment alone at decreasing mortality associated with YFV infection. Favipiravir at a dosage of 200 mg/kg/day was used as a positive control in this study, but we saw no significant difference in survival between this group and the placebo group. Typically, 60% mortality for YFV is observed in days 4-7 post infection for a hamster model. In this study, one hamster in the 200+40 treated groups died on day 13, which is abnormal and unexpected. Additionally, we saw lower-than expected mortality for the placebo-treated groups. All of these factors may indicate that infection was partially ineffective at the initiation of the study. If the virus was allowed to warm for too long or ineffectively administered intraperitoneally during infection, hamsters would not have been infected effectively, which may have skewed the results.

We saw consistent, sharp weight increase in the 200 mg/kg/day favipiravir-treated groups, 200 mg/kg/day favipiravir+20 mg/kg/day Compound X-treated groups, and non-infected control groups, indicating that the higher dosage of favipiravir alone or the combination treatment with the higher doses of Compound X and favipiravir were effective at preventing weight loss in infection (Figure 2). The similar 200+40 and 100+20 combination treatments also saw consistent weight increase throughout the study at a slower rate. Favipiravir alone or Compound X alone saw slower growth or decrease in weight, indicating these treatments are less effective at combating infection. The only significant difference we saw in total weight change was for the 200+40 combination treatment, indicating that this highest dosage of both favipiravir and Compound X was more effective than either treatment alone at treating YFV.

We saw no significant differences in the viremia or ALT levels across all groups. This puzzling result may be due to ineffective infection as previously mentioned. If hamsters were not effectively infected on day one of the study, many of the subjects would have not had significant

viremia or ALT levels until later in the study, far past the time when serum is collected to analyze either of these measures. The late weight loss and mortality of many groups supports the conclusion that infection was less than effective.

Despite these conflicting results, we are able to confidently conclude that the combination treatment of favipiravir with Compound X is more effective at treating yellow fever virus infection than either drug alone. This is especially true for the 200 mg/kg/day+40 mg/kg/day dosing, which significantly reduced mortality and weight loss across all groups treated. For multiple combination treatments, we observed zero percent mortality, a sharp reduction from the 60% in placebo and Compound X-only treated groups and 50% in the favipiravir-only treated groups. This result shows promise that in the future, these drugs may be used together to effectively combat yellow fever virus infection in humans.

Future studies should focus on ensuring infection is done properly at the time prescribed so that we can observe potential statistical differences in viremia and ALT levels. By ensuring proper infection technique, we can better analyze how these drugs interact with the virus across a reasonable, tested timeline. Additionally, future research should focus on more specific dosing for these two drugs in combination. With this study we have verified that the 200+40 dosage is non-toxic and effective at treating infection; in future studies, a smaller range of dosages (such as 150+30 to 250+50) should be used to exactly distinguish what is the smallest required dosing of these two compounds that still effectively combats sickness. With that knowledge in hand, these drugs can then be studied in larger in-vivo models and potentially implemented in human clinical trials in the future.

YFV is a familiar foe in the world today, and the lack of effective treatment options for those already infected demands our immediate attention. This study showed that a combination treatment of favipiravir with Compound X was effective at decreasing mortality and weight loss in an in-vivo model while avoiding toxicity due to the drugs themselves. This shows promise for development of an effective antiviral compound in the future for YFV infection.

	Favipiravir (mg/kg/day)	**Compound X (mg/kg/day)**	**Vehicle alone (mL/dose)**
Group 2	200	40	---
Group 4	100	40	---
Group 6	200	20	---
Group 8	100	20	---
Group 10	---	40	---
Group 12	---	---	0.1

Table 1: A summary of dosing for the toxicity study across all 6 non-infected groups. Favipiravir was administered twice a day orally, once in the morning, and once again approximately twelve hours later. Compound X was administered once a day in the morning intraperitoneally. Group 12 only received the vehicle these drugs were dissolved in as a control; the favipiravir vehicle was given orally twice a day, and the Compound X vehicle was given intraperitoneally once a day.

	Favipiravir (mg/kg/day)	**Compound X (mg/kg/day)**	**Vehicle alone (mL/dose)**
Group 2	200	40	---
Group 4	---	---	0.2
Group 6	---	---	---
Group 1	200	40	---
Group 3	100	40	---
Group 5	200	20	---
Group 7	100	20	---
Group 9	200	---	---
Group 11	100	---	---
Group 13	---	40	---
Group 17	---	---	0.2

Table 2: A summary of the dosing for the challenge study. Non-infected groups are indicated with even numbers and infected with odd. Favipiravir was given twice daily orally and Compound X was given once daily intraperitoneally in the doses listed. Vehicle was given to control groups by volume. Favipiravir vehicle was given twice daily orally, and Compound X vehicle was given once daily intraperitoneally.

Treatment	Dose (mg/kg/d), treatment initiation	Virus	Alive/total	Mean day of death ± SD	Mean wt. change(g) (4-7 dpi) ± SD	Viremia (4dpi)	ALT (6dpi)
Favipiravir+Compound X	200+40, beg 8 h	YFV	9/10	13.0 ± 2.5*	3.07 ± 2.68*	1.67 ± 0	68 ± 7
Favipiravir+Compound X	100+40, beg 8 h	YFV	8/10	12.5 ± 4.4	2.11 ± 2.81	1.95 ± 0.89	69 ± 10
Favipiravir+Compound X	200+20, beg 8 h	YFV	10/10	>21.0 ± 0**	6.28 ± 3.06	1.67 ± 0	73 ± 7
Favipiravir+Compound X	100+20, beg 8 h	YFV	10/10	>21.0 ± 0**	2.94 ± 3.84	1.75 ± 0.26	76 ± 7
Favipiravir	200, beg -4 h	YFV	8/10	9.5 ± 4.9	5.33 ± 2.05	2.10 ± 0.94	80 ± 12
Favipiravir	100, beg -4 h	YFV	6/10	10.0 ± 5.9	-3.08 ± 7.20	2.34 ± 1.69	112 ± 81
Compound X	40, beg -8 h	YFV	5/10	11.5 ± 5.4	0.94 ± 6.01	2.09 ± 0.95	73 ± 18
Vehicle	--	YFV	4/10	11.0 ± 5.4	4.40 ± 5.31	1.89 ± 0.47	82 ± 9
Favipiravir+Compound X	200+40, beg 8 h	Sham	5/5	>21.0 ± 0	3.22 ± 2.90	1.67 ± 0.0	66 ± 5
Placebo	--, beg 8 h	Sham	5/5	>21.0 ± 0	2.28 ± 2.70*	1.67 ± 0.0	68 ± 7
Normal Controls	--	Sham	5/5	>21.0 ± 0	4.36 ±4.61	1.67 ± 0.0	77 ± 10

Table 3: A summary of dosing for the challenge study across all 11 groups, compared to mean day of death, mean weight change, viremia, and serum ALT levels. It is shown here that the 200+40, 200+20, and 100+20 treatments all significantly decreased mortality as compared to the placebo. Additionally, mean weight change was significantly reduced in the 200+40 treatment. No difference was seen in serum ALT or viremia levels (**P<0.01, *P<0.05 as compared to placebo treatment).

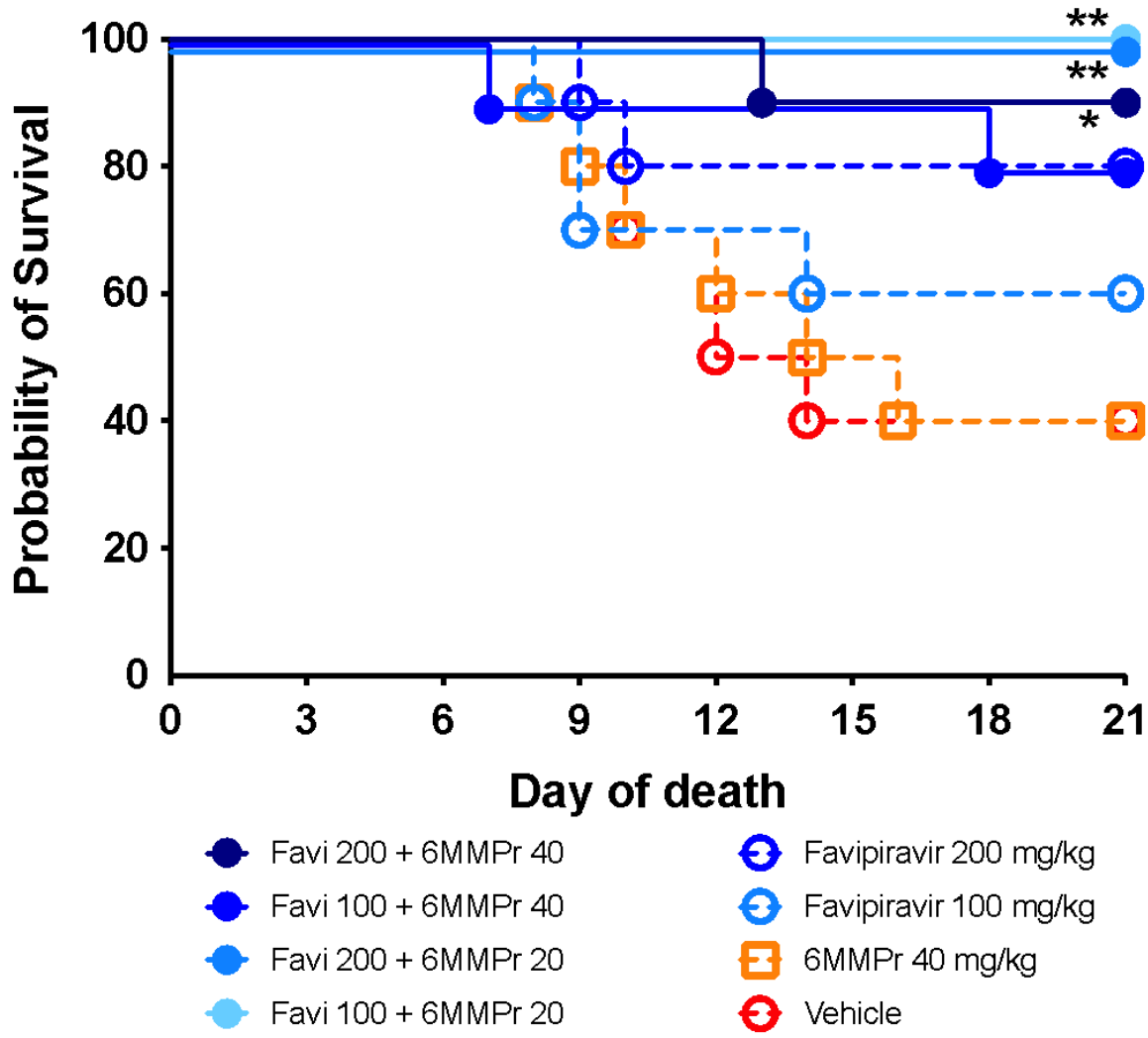

Figure 1: A comparison of mortality across the infected groups treated with differing dosages of the two drugs. Here we can see that the 100+20 and 200+20 treatments had 0% mortality overall and were statistically significant as compared to the placebo. Additionally, the 200+40 combination treatment had 10% mortality and was also statistically significant. This is compared to 60% mortality in the placebo-treated groups (**P<0.01, *P<0.05 as compared to placebo treatment).

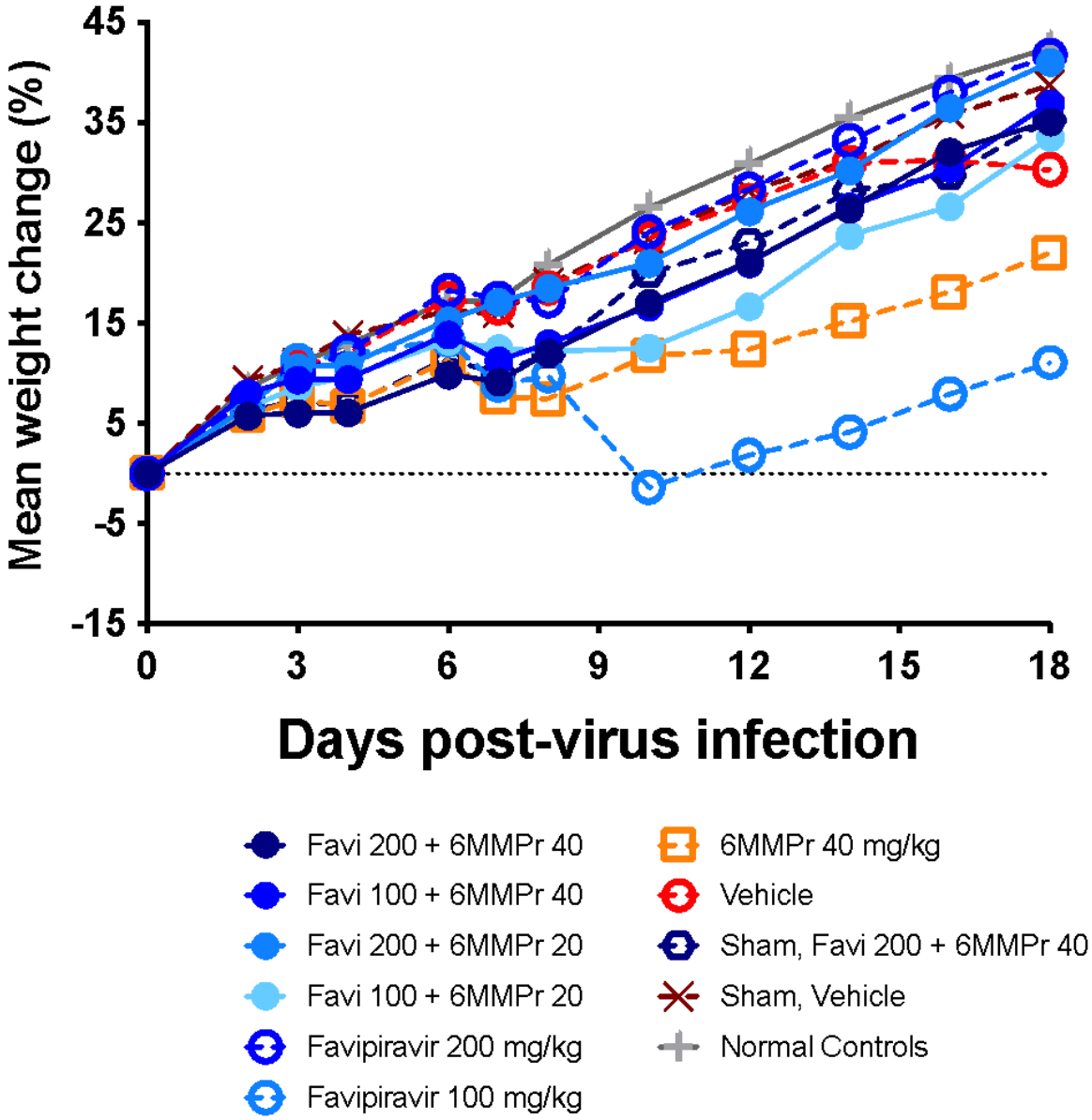

Figure 2: Mean weight change across all groups across the course of the study. Consistent weight increase was observed in all combination treatment groups as well as the higher dosage of favipiravir.

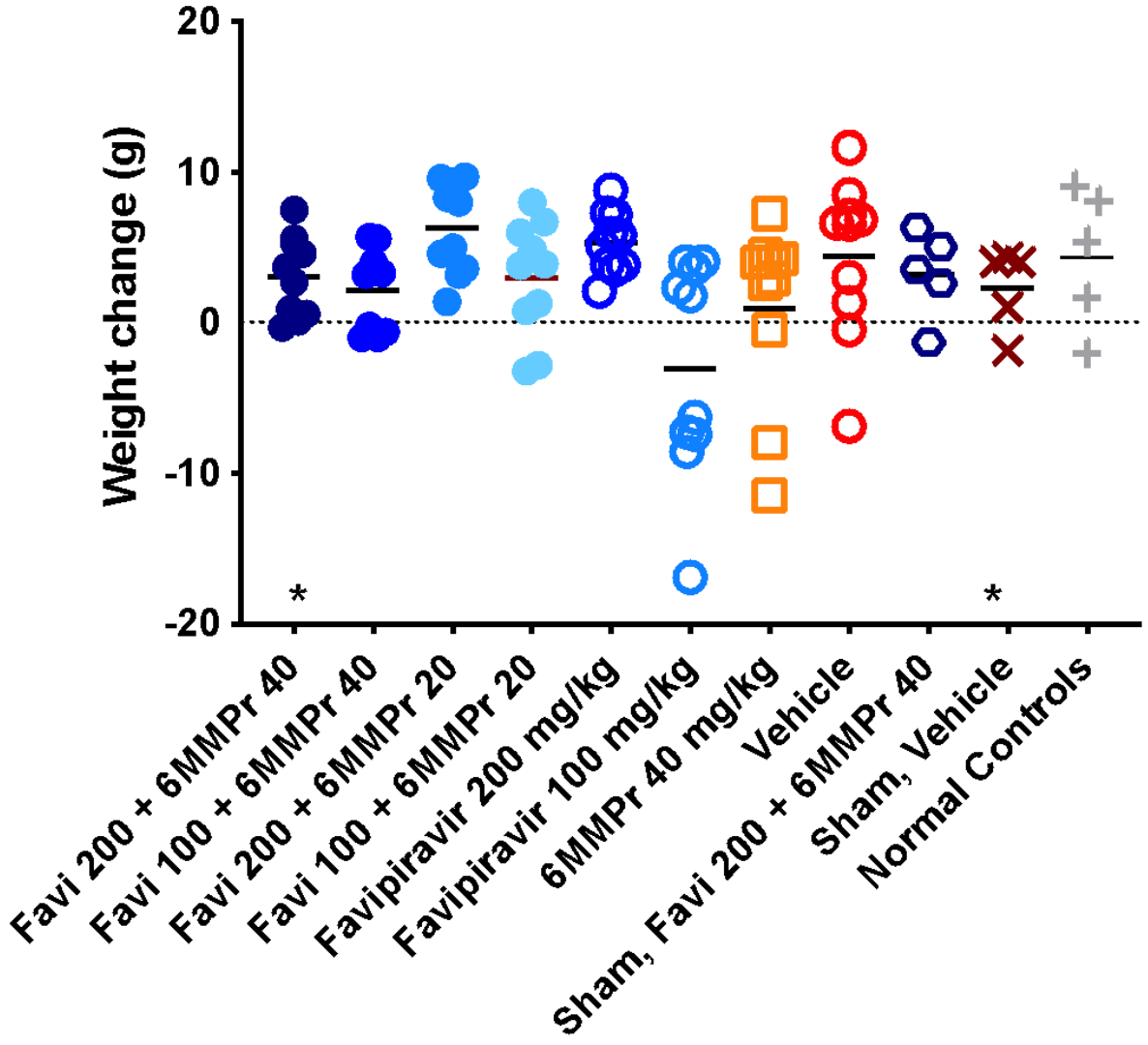

Figure 3: Mean total weight change across all groups. Here we can see that the dosage of 200+40 combination treatment significantly decreased weight loss as compared to the vehicle treatment for infected hamsters (**P<0.01, *P<0.05).

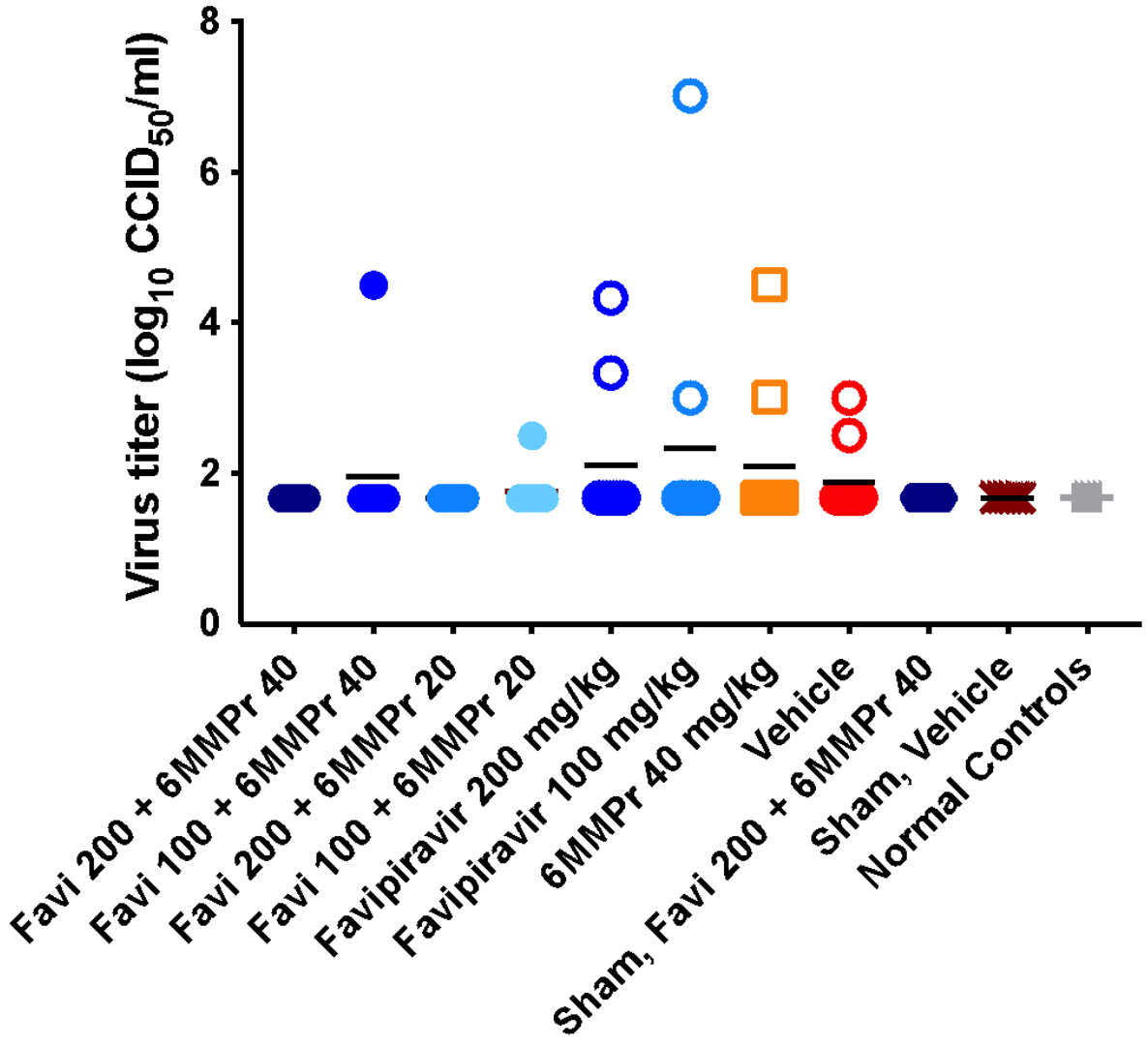

Figure 4: Viremia from serum collected on day four post infection. No significant difference can be observed between any of the treated groups. This may be due to an ineffective infection, as previously discussed.

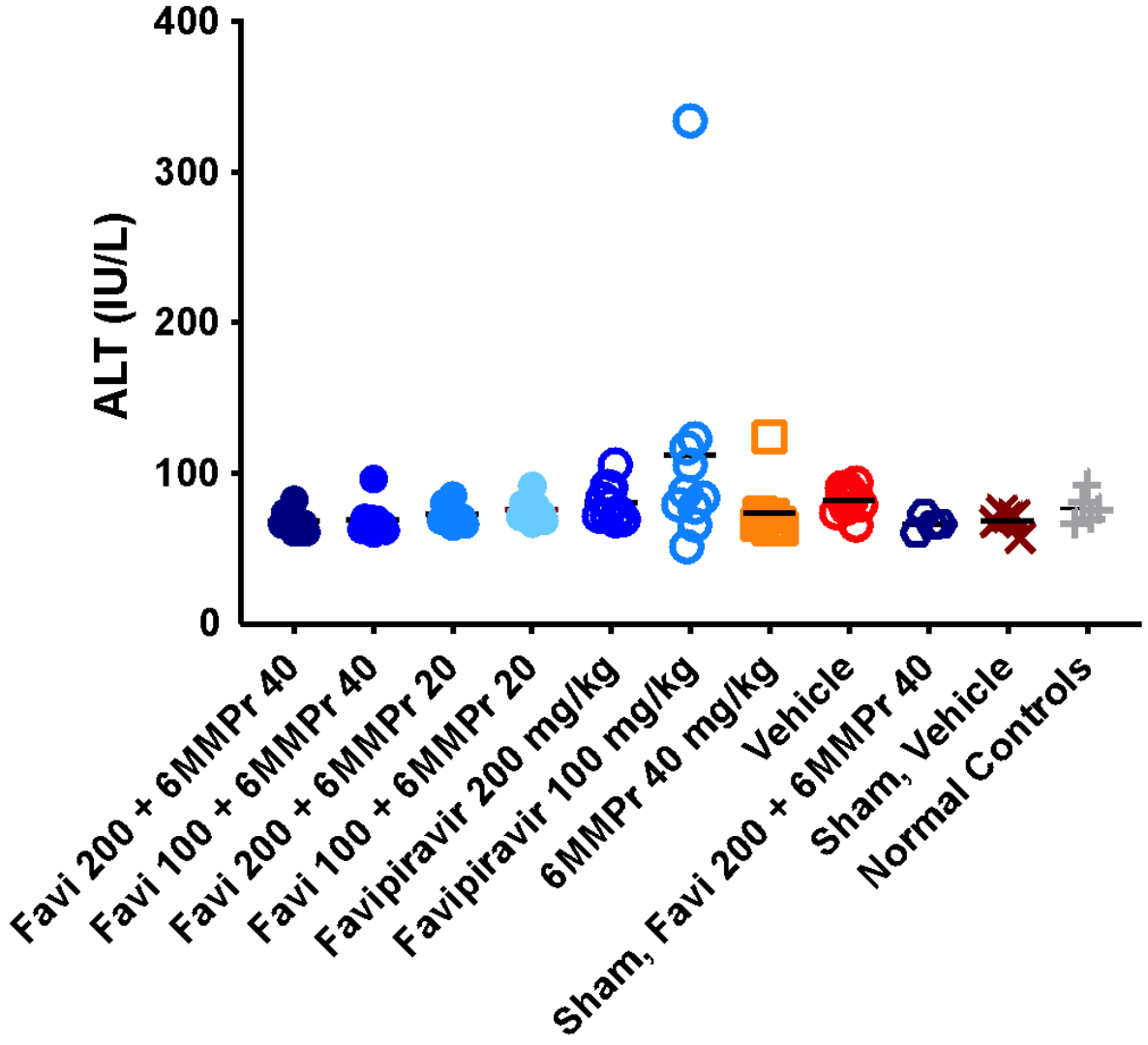

Figure 5: Alanine aminotransferase levels across all groups from serum collected on day six post infection. No significant difference can be observed between any of the treated groups. This may be due to an ineffective infection, as previously discussed.

Word count: 3554

Reflective Writing

My experience completing this capstone project has been extremely illuminating, challenging, and rewarding. This project allowed me to have an up-close, hands-on glimpse of how actual research takes place in an applicable setting. Throughout the course of the study, I learned how to design an experiment, present it to sponsors, effectively conduct tests, retrieve data, resolve problems that may arise, and evaluate and apply the end result of the research. These skills will be vital in my future career as a learner and a physician.

I began my college research journey in the Freeman lab, which studies the association of oxytocin and related hormones to different regions of the brains in rodents, coyotes, and humans. My time at this lab allowed me to develop proficiency at reading scientific literature. I also learned the skill of recognizing gaps in current knowledge and designing creative methods to potentially fill these gaps. This experience was invaluable in helping me to develop the skills necessary to transition to my current lab at the Utah State University Institute for Antiviral Research.

At the Institute for Antiviral Research, I worked for nine months as a research technician before attempting to begin my own project. During that time, I learned the ins and the outs of different tests we did in the *in vivo* department and how a typical study was designed. When I approached my mentor, Dr Justin Julander, about starting a project of my own, I was not aware of how much work and time went into the details of these processes. However, this experience has been invaluable at helping me to become a better student, researcher, and creative thinker.

I have always been interested in medicine, and so the idea of a study that tested a drug that could potentially be used to treat human disease in the future was especially appealing to

me. Yellow fever virus is a major worldwide health concern and finding effective solutions for infection has both short-term and long-lasting effects for vulnerable populations. Currently there is no effective antiviral compound developed for those infected with this disease, and mortality rates remain high. This research project held particular value for me because I understood that my findings could potentially be used in future studies to better develop drugs to treat those affected by this virus.

This project allowed me to establish a positive, beneficial relationship with my research mentor, Dr Justin Julander. Dr Julander has worked in the field of antiviral research for decades, and his understanding of the research process is unparalleled at Utah State University. I would meet weekly with Dr Julander to first discuss the background of yellow fever virus and research that has already been done in this field. We then began discussing potential gaps in knowledge, and he proposed this project to me as it had been presented to him by the sponsor. Throughout this entire process, Dr Julander was there to answer all of my tough questions, expound on my reasoning, help me with data analysis, and address any issues that arose. I hope to continue developing this relationship in the future to become a better researcher myself and contribute to Dr Julander's work.

Throughout this study, adjustments had to be made as different concerns arose. In the initial toxicity study, it came to our attention that the drug was not dissolving well in solution. We were able to find a way to heat and sonicate this compound so that it dissolved effectively for both the remainder of the toxicity and the entire challenge study. At the conclusion of the experiment, we were presented with some results we did not expect that conflicted with previous data. However, this gave me the opportunity to critically think back to our research process and identify potential errors that may have contributed to the conflicting results. This also gave me

the opportunity to identify ways in the future this study or other studies could improve the research process and better evaluate my research question. This project pushed and challenged me in multiple ways, but these skills of adapting to specific challenges and learning from error will be vital as a student, researcher, and physician in the future.

This project is applicable to a very specific discipline of antiviral research, but its format can be applied to a variety of experiences. Principally, this work was a collaboration; our sponsor developed this drug, tested it *in vitro*, then requested our help in testing it *in vivo*. In the future, this compound may go further in *in vivo* studies that will utilize other entities besides Utah State University. Our experiment required constant communication with the sponsor, and this experience helped me to learn how to effectively communicate with those on a research team. This skill can be applied to further disciplines beyond research: medicine, government, industry, and home life can all be improved by more effective, steady communication.

Science is interdisciplinary, and this project allowed me to deepen my understanding about how different fields contribute to each other's work. Though this project was very specifically focused on antiviral research, it drew on previous studies that involved designing, manufacturing, and testing a specific compound. Beyond that, in the future these results may be used not only in the field of viral studies, but also in medicine and treating underprivileged populations. Many poorer countries do not have effective access to an effective preventative vaccine for yellow fever virus, so the development of an accessible, cheap, effective antiviral drug may have far-reaching consequences beyond scholarly research.

This project has been crucial in helping me to develop my skills as a researcher and student, and these experiences will benefit me in my future career as a physician. Throughout

this project I learned how to work underneath a mentor, adapt to challenges, evaluate and learn from error, and collaborate with others. These are all beneficial skills that will help me to conduct further research, as well as work effectively as part of a team in the field of medicine in the future. I am grateful for the opportunity to complete this project and hope that my research can be continued to benefit those affected by this worldwide sickness impacting so many lives today.

Word count: 1039

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Hunter Stanger is currently a Senior studying Biology at Utah State University, with an emphasis in Human Biology and a minor in Chemistry. He has been involved with research for two years, beginning in the Freeman laboratory and currently working at the Institute for Antiviral Research. He has been involved in multiple research projects, including development of a protocol for the plaque reduction neutralization technique in *in vivo* antiviral studies as well as study of the location of oxytocin receptors in the brains of coyotes. He has served in various campus-wide leadership positions, including vice president and president of the Biology Undergraduate Student Association, committee chair of the USUSA pre-med outreach committee, and vice president of the USUSA MCAT club. He hopes to continue his research for the remainder of his time at Utah State University and plans on applying to medical school upon graduation in pursuit of his dream of becoming a physician.