Studying the effects of antibiotics on mitochondrial inhibitors

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Abstract

The usage of ivermectin (ivm) to treat COVID-19 is controversial. According to a study, ivermectin helped treat COVID-19, but clinical trials showed that ivermectin with the tetracycline-class antibiotic doxycycline overcame the effects of ivermectin to a certain degree[2]. The goal of this project is to understand why this occurs. Cell cultures were used as a model. Rather than overcoming the effects of ivermectin, tetracycline seems to prevent ivermectin from mitochondrial inhibition. This effect seems to occur when tetracycline is administered first, with ivermectin administered after one hour or more; the effect is prevented when ivermectin is administered, left for two hours, and then tetracycline is added and left for one hour.

Introduction

Hydroxycholoroquine (HCQ) has been shown to be a possible therapeutic in the treatment of COVID-19. It is an antimalarial drug, and part of its mechanism of action, according to studies, suggests that it sequesters protons in the intermembrane space of the mitochondria. Some data suggests that COVID-19 hijacks the mitochondria and sequesters protons, destroying the proton gradient required for ATP production and causing eventual cell death[1]. In this way, (HCQ) could show therapeutic effects by sequestering the protons in a gradual fashion. HCQ is therefore used as a control.

The study showing that ivm+doxycycline shows lower viral clearance than ivm alone presents the following graph[2]:

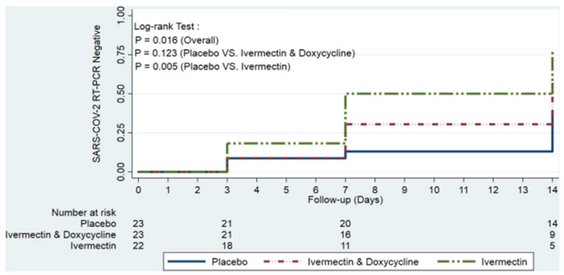


Figure 1. Cumulative viral recovery estimates in the overall study population.

Ivm is an antiparasitic. Doxycycline is an antibiotic often used to prevent secondary infection in diseases that compromise lung function.

Hypothesis

Ivermectin acts on the mitochondria to inhibit ATP synthesis, and adding tetracycline overcomes mitochondrial inhibition.

Methods and Materials

- Using tetracycline (tet) as the antibiotic, varying concentrations.
- Varying concentrations of ivermectin (ivm).
- Hydroxychloroquine (HCQ) used as a control (various concentrations).
  - Availability
  - Appears to target mitochondria in a different way than ivermectin
- See if antibiotic overcomes both mitochondrial inhibitors (HCQ and ivm) that function differently.
- Use of Cell Titer Glo assay:
  - 15k cells in 100 microliters media per well in a 96-well polystyrene assay plate. Add drugs and incubate for two hours.
  - Add CTG reagent, causing cell lysis and ATP release, used to generate luminescence. Measure cell viability based on ATP production.

Results

Figure 1. Various concentrations of tet with constant HCQ and ivm concentrations on h293 cells (kidney epithelial cancer cells)

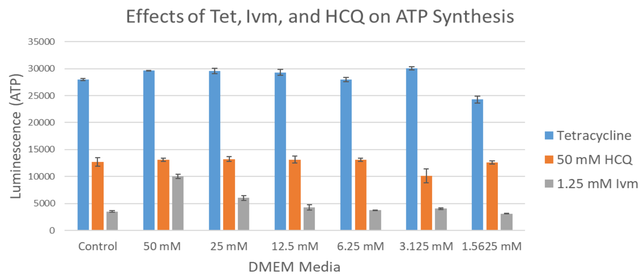


Figure 2. Various concentrations of ivm while adding 50 mM tet (most effective) on a549 cells (lung epithelial cancer cells)

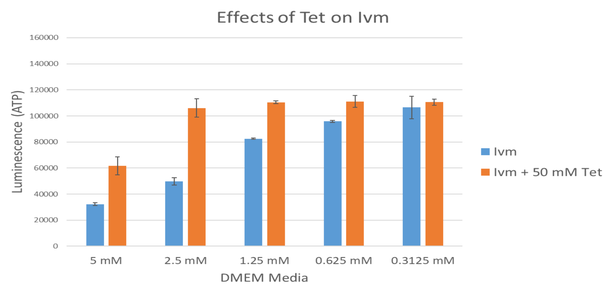
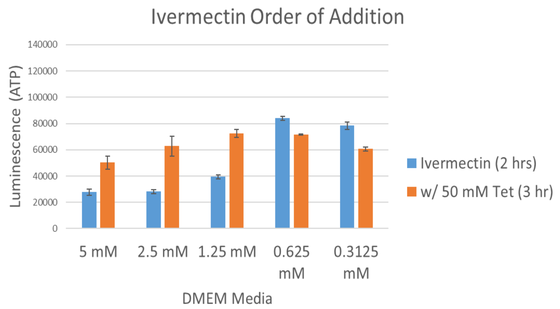


Figure 3. Order of addition, tet for 1 hour then add ivm, wait for 2 hours



Discussion and Conclusions

Figure 1 shows that tet affects ivm but not HCQ. Figure 2 shows inhibition decreases (signal increases) with decreasing concentration of ivm, and tet affects ivm to a certain extent (i.e. maximum signal). Order of addition with ivm 1 hour, then tet 2 hours (not shown) shows time dependence of ivm, but no statistical difference between ivm and ivm+tet conditions. Figure 3 shows less inhibition from ivm with addition of tet, proving that instead of overcoming inhibition, tet is probably *preventing* ivm from mitochondrial inhibition. However, tet could be reestablishing a switch to glycolysis instead to compensate for inhibited mitochondria.

Future Directions

- See whether tet is re-establishing switch to glycolysis *or* preventing ivm mitochondrial inhibition.
- Finding a class of antibiotics that has the same preventative measures as doxycycline without affecting ivermectin.
  - Maximize efficiency of treatment with protection of antibiotic.

Literature Cited

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2. Ahmed, S., Karim, M. M., Ross, A. G., Hossain, M. S., Clemens, J. D., Sumiya, M. K., Phru, C. S., Rahman, M., Zaman, K., Somani, J., Yasmin, R., Hasnat, M. A., Kabir, A., Aziz, A. B., & Khan, W. A. (2021). A five-day course of ivermectin for the treatment of COVID-19 may reduce the duration of illness. International Journal of Infectious Diseases, 103, 214–216. https://doi.org/10.1016/j.ijid.2020.11.191
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