Screening Warrior Hops for Inhibitors of Cancer Cell Metabolism

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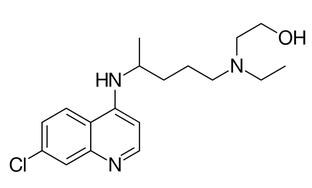
Abstract

The purpose of this project is to examine the effects of components of Warrior Hops on cancer cell metabolism to better understand the mechanism of infection for SARS-CoV-2. In h293 cells (human embryonic kidney cells that metabolically mimic cancer cells), several fractions have been obtained that showed mitochondrial inhibition and the inhibition of ATP production in cells similarly to hydroxychloroquine, which acts as the positive control. This project is still in progress and will provide a better understanding of the mechanism of infection for COVID-19 on the cellular level.

Introduction

Hydroxychloroquine is an anti-malarial drug for which its use to combat COVID-19 has been controversial. Previous research has shown that the unique structure of hydroxychloroquine, which contains amine groups that can be protonated and deprotonated at physiological pH, inhibits the normal metabolism of mitochondria in cells by sequestering protons, thereby reducing the gradient necessary to produce ATP. Not only is hydroxychloroquine able to sequester protons from the intermembrane space, but it is also able to enter the intermembrane space in a mechanism that is not well defined. Amine groups that can be protonated or deprotonated at physiological pH are common in plant steroids, so I am evaluating potential components of the herb Warrior Hops that can produce a similar or greater effect on mitochondrial metabolism than hydroxychloroquine, while potentially avoiding the detrimental side effects that make hydroxychloroquine so controversial in treating COVID-19. Warrior hops is an herb used in beverages and in herbal medicine and its fractions of various polarities are screened on h293 cells and the purified mitochondria of these cells for metabolic inhibition.

Warrior Hops



Hydroxychloroquine

Hypothesis

There are components in Warrior hops that inhibit mitochondrial metabolism, thereby reducing the amount of ATP produced by the cell.

Methods and Materials

Separation

- Collected 13 eluates in increasing polarity (from hexanes to methanol) using a packed silica column and resuspended the dried eluates in DMSO

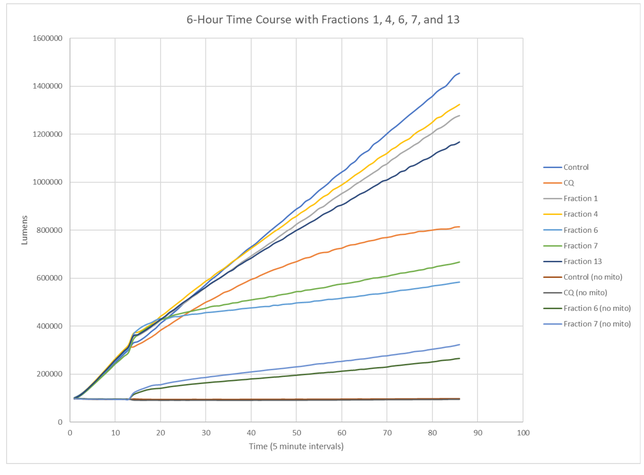
Cell Screening

- Preincubate h293 cells in L-15 media +/- 20 mM glucose (~10,000 cells/well) for 1 hour
- Add 2 µL of fraction and incubate for 1 hour
- Run Cell Titer Glo Assay to measure ATP

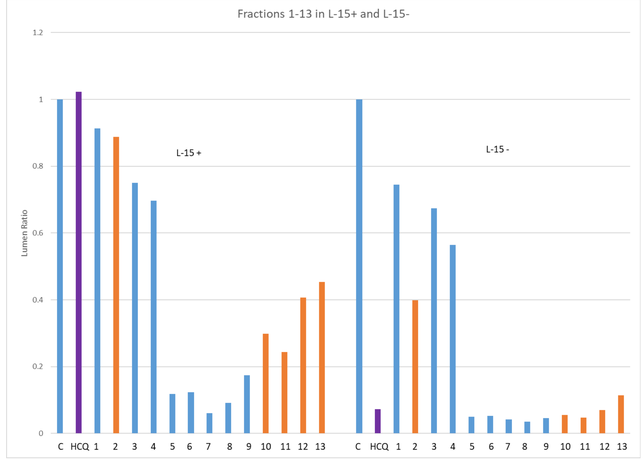
Mitochondrial Screening

- Plate purified mitochondria in Locke's buffer + 1 µg/mL BSA + 2 µL/well Cell Titer Blue
- Run kinetics in the plate reader for 1 hour using Cell Titer Blue assay to establish baseline
- Add 2 µL of fraction and run kinetics for 6 hours

Results



Fractions 6 and 7 showed greater mitochondrial inhibition than hydroxychloroquine, the positive control.



Fractions 6 and 7 also showed inhibition in the cell screen.

Discussion and Conclusions

Fractions 6 and 7, which were eluates of solvent 6 and 7 (hexane and ethyl acetate mixture and ethyl acetate, respectively), showed cellular inhibition of ATP production and inhibition of mitochondrial metabolism as shown in both the cell and mitochondrial screenings. These fractions underwent further separation via silica column chromatography, and this experiment was repeated, confirming that there are elements in these fractions that inhibited cellular ATP production and mitochondrial metabolism.

Future Directions

- Repeat using larger amounts and different brands of hops
- ATP controls
- Cell Titer Blue cell screen
- Vary media: DMEM +/- 2DG
- Quantify purified mitochondria
- Further column chromatography
- GC analysis
- Varying the solvent used during the cellular and mitochondrial screens

Literature Cited

Sheaff, R. J. (2020, January 1). A new model of SARS-COV-2 infection based on (hydroxy) chloroquine activity. bioRxiv.

Cadenas, S. (2018, May 31). Mitochondrial uncoupling, ROS generation and cardioprotection. Pubmed

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