Lycorine effective against Yellow Fever Virus

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Introduction

- Yellow fever virus (YFV)
- Hemorrhagic disease
- Mosquito-borne
- Vaccine available but no treatment
- Tested Lycorine against YFV

Chikungunya Virus (CHIKV)

- Causes arthritic and joint pain
- Mosquito-borne
- No treatment available
- Tested Silymarin against CHIKV

Methods

- Cytopathic effect (CPE) inhibition assay
  - Lycorine and Silymarin were purchased as solid compound from Sigma-Aldrich Chemical Company.
  - Compounds are prepared at 2X the desired starting concentration in appropriate diluent (Saline for Lycorine and 10% DMSO for Silymarin).
  - Half-log dilutions of the compound are prepared in cell culture medium
  - The compound dilutions are added to cell monolayers (Vero 76 or HUH7 cells) in a 96-well plate (within the orange/green and yellow boxes in Fig. 3).
  - An equal volume of virus (17D YFV or LR2006 OPY1 CHIKV) was added immediately after addition of compound at a concentration that would result in complete CPE of virus controls (in the orange, red, and green boxes on Fig. 3).
  - Plates are observed for CPE visually and results recorded.
  - After visual read, plates are stained with neutral red (NR) for a dye uptake assay to determine:
    - The 50% effective concentration (EC50) or concentration of the compound needed to prevent 50% of the cells from dying (Orange and green boxes in Fig. 3).
    - The 50% inhibitory concentrations (IC50) or concentration of the compound that results in toxicity to the 50% of the cells (Yellow boxes in Fig. 3).
    - The selective index (SI) is calculated by dividing IC50/EC50 and gives an indication of efficacy.
    - A compound with an SI>10 is considered active and warrants further testing.

- Virus yield reduction (VYR) assay
  - Plates were prepared as above.
  - When CPE was observed, cells were frozen.
  - Virus infected wells with the same concentration of compound were pooled and titered on HUH7 cells.
  - CPE was recorded at an appropriate time depending on the virus used.
  - The EC90 of the compound, or concentration of compound required to reduce virus by 1 log10, was determined.

- Controls included Infergen, 6-Aza, Ribavirin

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Results

- Lycorine (Active)
  - CPE results
    - EC50= 0.33 µg/ml
    - IC50= 58 µg/ml
    - SI=180
    - EC90= 0.42 µg/ml
    - New SI= 136
  - Silymarin (Marginally Active)
    - CPE results
      - EC50= 87 µg/ml
      - IC50= 190 µg/ml
      - SI= 2.2

Discussion

- Lycorine was highly active against YFV, while Silymarin was marginally effective against CHIKV.
- Potential problems with the cell controls in HUH7 cells:
  - Not a confluent monolayer to begin with.
  - This could have skewed our results yielding a higher SI than is correct (we will repeat the study to verify results).

Future Research

- An SI of >10 warrants further testing.
- Silymarin does not warrant further testing.
- Lycorine warrants further testing.
  - Repeat CPE inhibition assay
  - Repeat VYR assay
  - Test in animal model

References

Photos:
2) http://www.chemspider.com/Chemical-Structure.23089618.html
3) http://www.thurnscoe-exotics.co.uk/syrian-hamsters