**Lycorine effective against Yellow Fever Virus**

* Ivy Binks, Justin Julander  
* Institute for Antiviral Research, Utah State, Logan, Utah, USA  
* Contact: ivy.binks@aggiemail.usu.edu

---

**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

**Acknowledgements**

Justin Julander  
Joseph Evans, Nate Clyde, Sean Wright  
The NIH for funding  
The Institute for Antiviral Research

---

**Results**

- **Lycorine** (Active)  
  - CPE results  
    - EC50=.33 µg/ml  
    - IC50= 58 µg/ml  
    - SI=180  
    - EC90=.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