**In Vitro Efficacy of Cidofovir and Acyclovir against Frog Virus 3**

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**Objective**
Evaluate the *in vitro* efficacy of antiviral drugs Acyclovir and Cidofovir to reduce ranavirus titers in cell cultures.

**Background**
- Frog virus 3 is a *Ranavirus* in the family *Iridoviridae*
- Large double-stranded DNA viruses
- Significant die-offs in wild and captive amphibian, reptile, and non-tetrapod fish populations
- Little is understood about chemotherapeutics for iridoviral diseases
- Acyclovir, a chain terminator prodrug, treats herpesvirus infections
  - Requires viral phosphorylation to become active
- Cidofovir is a chain terminator drug that does not require viral activation

**Methods**
- *Terrapene* heart cells (TH-1) grown to confluency in 10% complete media in 24 cm² well cell culture plates, incubated at 28°C in 5% CO₂
- Isolated and partially characterized ranavirus from a Burmese Star tortoise used to inoculate cells at starting concentration of 10⁵ TCID₅₀/ml
- Antivirals added
  - Acyclovir diluted into 25 mL cell culture media in 0, 0.2, 1, 5, 10, and 25 µg/ml concentrations
  - Lipid-encapsulated cidofovir diluted into 25 mL cell culture media in 0, 0.2, 1, 5, and 10 µg/ml concentrations
- Cells allowed to incubate for four days
- Wells evaluated for cytopathic effects (CPE)
- Virus titers estimated using a tissue culture infectious dose (TCID₅₀) assay

**Results**
- After four days, all wells with various concentrations of acyclovir showed CPE
- While there was a trend observed towards a decreasing titer with increasing acyclovir concentrations, the decrease was not statistically significant (p=0.287)
- Preliminary investigations demonstrated acyclovir to be insufficient at completely inhibiting viral replication
- Cidofovir testing is still underway

**Discussion**
- While this study did not find acyclovir to be effective for ranavirus, doses higher than 25 µg/ml were not evaluated
  - Nephrotoxicity and bone marrow suppression seen with acyclovir in cats
  - Anorexia seen in a pharmacokinetic study in box turtles at 40 mg/kg PO
- Acyclovir has been found to be 50% effective against feline herpesvirus-1 at a concentration of 56 µg/ml warranting more studies at higher concentrations
- If effective, cidofovir will require further in vivo safety and pharmacokinetic studies.

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**Diagram**
Effect of increasing concentrations of acyclovir on the TCID₅₀ of *Terrapene* cells inoculated with the Burmese star tortoise isolate.