Use of 5-(Perylen-3-yl)Ethynyl-arabino-Uridine (aUY11) to inactivate Rift Valley fever virus and preserve neutralization epitopes.

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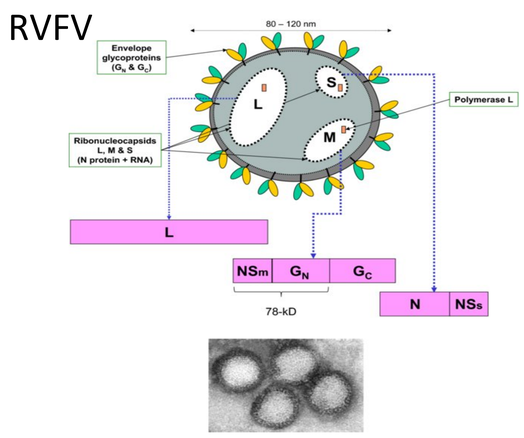
Abstract

Rift Valley fever (RFV) is a mosquito-borne disease, endemic to Africa, and known to cause abortion in animals and hemorrhagic fever in humans. All enveloped viruses require the fusion of viral membranes with cellular membranes to enter into and replicate inside the host cell. These viruses have a universal objective: find the cell receptors that match the viral attachment proteins and use those to gain access to the cell membrane. The use of aUY11, a monosaccharide (sugar)-based compound, has previously shown to effectively use a biophysical approach in the inhibition of viral fusion of other enveloped viruses[1]. In this study, we attempt to use aUY11 to inhibit the fusion of Rift Valley fever virus (RVFV) *in vitro* as a primary step in the creation of an FDA-approved vaccination for humans and animals to protect against often-lethal RVFV infection. We show here that aUY11 inhibits viral replication in Vero 76 cells and therefore has potential as an RVFV vaccine.

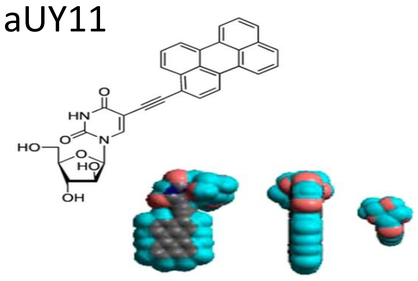
Introduction

- Rift Valley fever is an acute viral disease teratogenic and lethal in many livestock animals such as cattle, goats, and sheep.
- RVF is endemic to sub-Saharan Africa, and major outbreaks of RVF have recently occurred in Egypt, the Arabian Peninsula, and Madagascar[2], demonstrating its significant potential to spread to new geographical areas.
- Transmission occurs largely via mosquitoes, but can also be spread through the milk of an infected animal or contact with infected blood[3].
- For hospitalized persons diagnosed with RVFV, fatality rates can reach 10-20%[4].
- No FDA approved antivirals currently exist for humans.
- Wild type (WT) RVFV is known to the CDC and USDA to pose a severe threat to human and animal health (known as a select agent), and experiments using this strain require working in a biosafety lab 3+ (BSL-3+)[5].
- For the current study, we examined the use of aUY11 to inactivate the fusion of MP-12, an attenuated RVFV strain that can be handled in a BSL-2 lab[5].
- aUY11 is a member of the family of rigid amphipathic fusion inhibitors (RAFI), compounds that targets viral membranes to prevent virus-to-cell fusion
- The use of aUY11 to block viral fusion at cell membranes has been proven in other enveloped viruses, such as Hepatitis C, Herpes virus, and Influenza[1].

RVFV



aUY11



Objectives

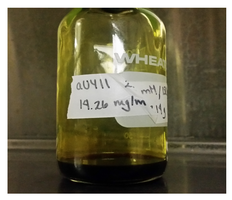
- Determine whether aUY11 can completely inactivate MP-12 vaccine strain of RVFV
- Determine immunogenicity of aUY11-inactivated MP-12
- Evaluate efficacy of aUY11-inactivated MP-12 in small animal models of Rift Valley fever

Materials and Methods

- **Cells and culture conditions.** Vero 76 (African green monkey kidney) cells were purchased from American Type Culture Collection (ATCC) and cultured in flasks. Cells were grown in T-75 and T-150 flasks at 37 ^{o}C and 5% CO_2 in MEM media with 10% Fetal Bovine Serum.
- **Virus strain.** MP-12 strain obtained from Dr. Tetsuro Ikegami at UTMB in Galveston, Texas. The MP-12 strain of RVFV was propagated in Vero 76 cells.
- **aUY11.** aUY11 obtained from Dr. Luis Schang at the University of Alberta. Several concentrations of aUY11 (20uM, 25uM, 50uM, 75uM, and 100uM) were tested to inactivate MP-12.
- **Time-sensitive treatments.** aUY11 was added to the virus stock in 1 hour increments over a period of six hours inside a 39 ^{o}C and 5% CO_2 incubator to allow time for the aUY11 to intercalate into the individual virion membranes.
- **aUY11 MP-12 attachment.** aUY11 and RVFV were placed on stir plates and a 3-D rotator at 39 ^{o}C and 5% CO_2.
- **Incubation.** The cell culture plates were incubated for several days and monitored for cytopathic effects (CPE).

Experiment Flowchart

Vero 76 cells cultured in T-75 and T-150 flasks

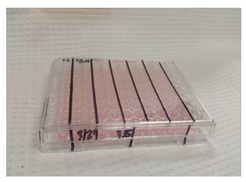


aUY11 applied to RVFV stock (below) to begin inactivation

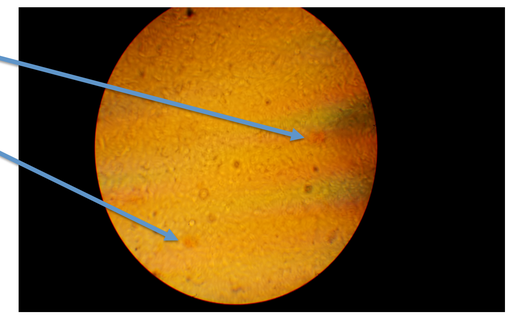
seeding in 96-well plates for infection with aUY11-inactivated RVFV

RVFV, treated with aUY11 and diluted, added to plates and incubated at 39 ^{o}C in 5% CO_2

Cells monitored for aUY11 toxicity and infection by RVFV



Results



- At concentrations between 50uM and 75uM, aUY11 has exhibited inhibition of viral infection of RVFV cell culture.
- For all concentrations, inactivation of virus using a stir plate proved more effective than a 3-D rotating plate
- aUY11 is not toxic to cells and has no effects on cellular functions, such as cell growth at 75uM and below
- No CPE was observed at concentrations of 75uM after four days.

Discussion

aUY11 targets enveloped viruses. In this study, aUY11 shows partial inactivation of RVFV at concentrations between 50uM-75uM. Further study to determine optimal inactivation period and conditions, and ultimately the potential for use *in vivo* as a vaccine candidate is required. Our plans are to optimize the vaccine preparation *in vitro*, and then vaccinate animals six weeks before infection (prime) and three weeks later (booster) to evaluate immunogenicity and efficacy in rodent models.

References:

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Acknowledgements

Supported by the Institute of Antiviral Research and the Gowen Lab