Treatment of Yellow Fever Virus with a Guanosine Analog, Compound X, in a Hamster Model

Abbie E. Weight,[1,2] Justin G. Julander[2]

[1]*Department of Biology, Utah State University* [2]*Institute for Antiviral Research, Utah State University, Logan, Utah, USA*

Abstract

Yellow fever virus (YFV) continues to cause significant global morbidity and mortality, yet no approved antiviral treatments are available for the disease. A recently developed guanosine analog, Compound X, has shown promise in vitro against multiple flaviviruses. The objective of the present study was to evaluate the in vivo efficacy of this compound against YFV in a hamster model.

Varying concentrations of Compound X were tested in different groups of YFV-infected hamsters, and weight and mortality were monitored throughout the course of the study. Serum samples were collected at 4 days post infection (dpi) for virus titer and 6 dpi for alanine aminotransferase (ALT) analysis. Compound X improved survival among hamsters infected with YFV in a dose-dependent manner. This compound warrants further investigation as a promising antiviral treatment for YFV.

Introduction

- Yellow fever virus (YFV) is a serious flaviviral infection endemic to tropical and subtropical regions of South America and Africa.
- Although an effective vaccine is available, YFV still causes significant global morbidity and mortality, and there are no approved antiviral treatments available.
- There are no approved antiviral treatments for YFV.
- Nucleoside analogs are an important class of antivirals that interfere with viral replication.
- Compound X, a novel guanosine analog, has shown promise in vitro as a treatment against flaviviruses and is tested in vivo in the present study.

Objectives

- Evaluate the efficacy in vivo of Compound X in a hamster model of YFV
- Compare the efficacies of different concentrations of Compound X

Materials and Methods

Animals: 92 female Syrian golden hamsters were used. Hamsters were block-randomized by weight to experimental groups and individually marked with ear tags.

Virus: Yellow fever virus (Jimenez hamster-adapted strain). A challenge dose of 200 $CCID_{50}$ per hamster (approximately 6 X the LD_{50} in hamsters) was administered via bilateral i.p. injection of 0.1 ml.

Test agent: Compound X was provided by sponsor and prepared according to sponsor directions.

Infectious cell culture assay: Virus titer was quantified using an infectious cell culture assay where a specific volume of serum was added to the first tube of a series of dilution tubes. Serial dilutions were made and added to Vero cells. Ten days later, cytopathic effect (CPE) was used to identify the end-point of infection. Four replicates were used to calculate the 50% cell culture infectious doses (CCID50) per mL of plasma or gram of tissues.

Serum aminotransferase assays: Serum was collected via ocular sinus bleed on 6 days post-virus infection (dpi). ALT (SGPT) reagent (Teco Diagnostics, Anaheim, CA) was used, and the protocol was altered for use in 96-well plates. Briefly, 50 µl aminotransferase substrate was placed in each well of a 96-well plate, and 15 µl of sample was added at timed intervals. The samples were incubated at 37˚C, after which 50 µl color reagent was added to each sample and incubated for 10 min as above. A volume of 200 µl of color developer was next added to each well and incubated for 5 min. The plate was then read on a spectrophotometer, and aminotransferase concentrations were determined per manufacturer's instructions.

Experiment Design: Hamsters were challenged with YFV via bilateral IP injections. Animals were treated orally with Compound X or intraperitoneally with Ribavirin as a positive control. Treatment was initiated -4 hours or 2 dpi and continued twice daily for 7 days. Hamsters were monitored for mortality from 0-21 dpi. Individual weights were recorded 0 dpi and daily from 3-18 dpi. Serum was collected on 4 and 6 dpi for analysis of virus titer and ALT, respectively.

Statistical analysis: Survival data were analyzed using the Wilcoxon log-rank survival analysis and all other statistical analyses were done using one-way ANOVA using a Dunnett multiple comparison (Prism 5, GraphPad Software, Inc).

Results

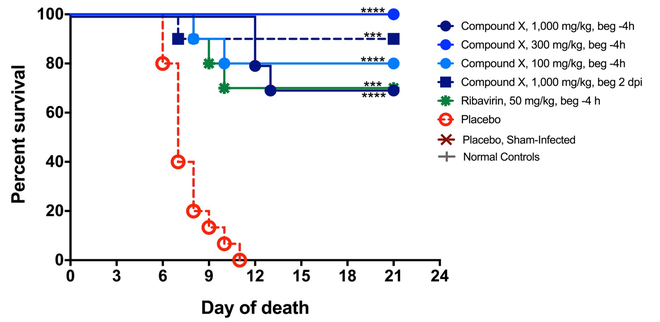

Figure 1. *Mortality curves of Syrian golden hamsters after treatment with Compound X or ribavirin and challenge with YFV (****P<0.0001, ***P<0.001 as compared to placebo treatment).*

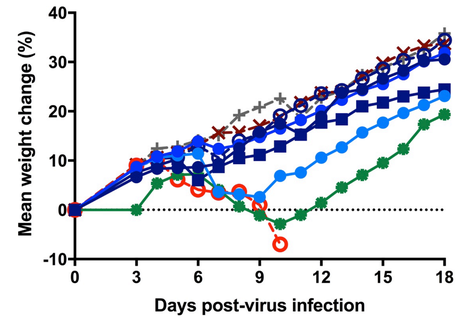

Figure 2. *Average percent weight change of animals challenged with YFV and treated with Compound X or ribavirin.*

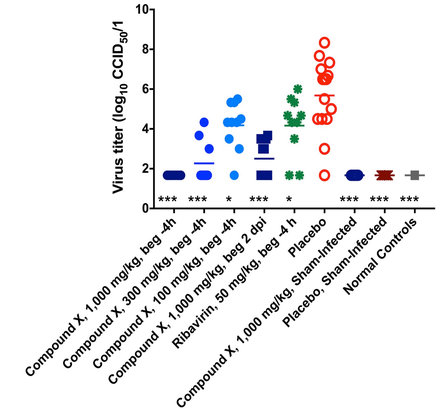

Figure 3. *Viral titers from serum collected 4 dpi from animals treated with Compound X or ribavirin (***P<0.001, **P<0.01, *P<0.05 as compared to placebo treatment).*

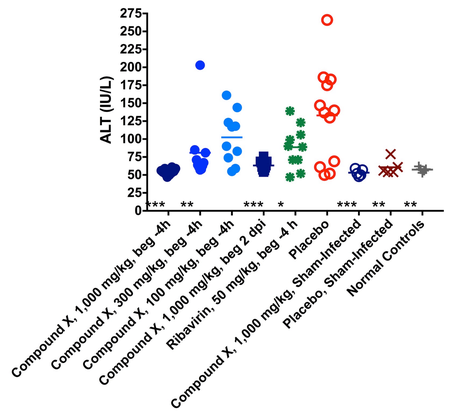

Figure 4. *Serum ALT levels (as a measure of liver damage) from samples collected 6 dpi from animals infected with YFV and treated with Compound X or ribavirin (***P<0.001, **P<0.01, *P<0.05 as compared to placebo treatment).*

Conclusions

- All concentrations of Compound X showed significant improvement in hamster mortality, weight change, ALT levels, and viremia as compared with placebo in the treatment of YFV.
- Higher concentrations of Compound X showed greater improvement in disease parameters.
- Overall, Compound X shows promise as an antiviral treatment for YFV in a hamster model and thus warrants further investigation.

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