

Development of FGI-106 as a broad-spectrum therapeutic with activity against members of the family *Bunyaviridae*

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Abstract: The family *Bunyaviridae* is a diverse group of negative-strand RNA viruses that infect a wide range of arthropod vectors and animal hosts. Based on the continuing need for new therapeutics to treat bunyavirus infections, we evaluated the potential efficacy of FGI-106, a small-molecular compound that previously demonstrated activity against different RNA viruses. FGI-106 displayed substantial antiviral activity in cell-based assays of different bunyavirus family members, including Asian and South American hantaviruses (Hantaan virus and Andes virus), Crimean-Congo hemorrhagic fever virus, La Crosse virus, and Rift Valley fever virus. The pharmacokinetic profile of FGI-106 revealed sufficient exposure of the drug to critical target organs (lung, liver, kidney, and spleen), which are frequently the sites of bunyavirus replication. Consistent with these findings, FGI-106 treatment delivered via intraperitoneal injection prior to virus exposure was sufficient to delay the onset of Rift Valley fever virus infection in mouse-based models and to enhance survival in the face of an otherwise lethal infection. Altogether, these results suggest a potential opportunity for the use of FGI-106 to treat infections by members of the family *Bunyaviridae*.

Keywords: Rift Valley fever virus, bunyavirus, hantavirus, antiviral, therapeutic

Introduction

The family *Bunyaviridae* contains a diverse group of animal-infecting viruses to include the *Orthobunyavirus*, *Phlebovirus*, *Nairovirus*, and *Hantavirus* genera. These viruses are mainly arthropod-borne except for the hantaviruses, which are spread by the aerosolization of rodent excreta. The bunyaviruses can cause a wide range of illness in humans ranging from mild asymptomatic infection to a form of viral hemorrhagic fever, pulmonary disease, or fatal encephalitis. At least one genera within the family *Bunyaviridae* is endemic to almost every region of the world.¹ Additionally, many of the bunyaviruses, such as Rift Valley fever virus (RVFV), are considered a potential biothreat agents and are characterized as Category A, B, or C agents by the United States National Institute for Allergy and Infectious Diseases (US NIAID).

The *Hantavirus* genus contains viruses, which cause either hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS). The viruses that cause HFRS are found throughout Europe and Asia while the viruses causing HPS are found in North and South America. The prototypic HFRS virus is Hantaan virus (HTNV), while the HPS prototype is Andes virus (ANDV). The *Nairovirus* genus includes Crimean-Congo hemorrhagic fever virus (CCHFV) which is endemic in Europe, Africa, and Asia. Significant fatality is observed in up to 50% of people that are hospitalized due to infection with CCHFV. The illness is hepatic in manifestation

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and results in systemic hemorrhages. The prototype of the *Orthobunyavirus* genus is La Crosse virus (LACV), an encephalitic virus found throughout North America that is spread when the host is fed upon by an infected mosquito. The genus *Phlebovirus* contains many viruses that cause significant disease in animals and humans and RVFV is an important emerging arthropod-borne disease within this genus. Infection with RVFV leads to abortion and high mortality in domestic animals, while humans can become incidentally infected.² Humans may become infected when fed upon by infected mosquitoes (*Aedes* and *Culex* species mosquitoes appear to be the principal vectors) or by contact with tissues, blood, or fluids from infected animals. Typically, upon infection, humans present with nonspecific influenza-like symptoms, but severe cases may involve hepatitis, hemorrhaging, encephalitis, and retinitis. Mortality rates range from 1%–3%, but increase drastically with the severity of symptoms. Human cases have been reported in much of Africa, Saudi Arabia, and Yemen, with recent outbreaks in Kenya during 1997–1998 and 2006–2007.³

As discussed above, the members of the *Bunyaviridae* family can cause a wide range of illnesses, all of which have potential for unfavorable outcomes. Currently, there is a lack of safe and effective therapeutics to treat bunyavirus infection. Ribavirin, a nucleoside analog, has antiviral activity against the hantaviruses, phleboviruses, nairoviruses, and the orthobunyaviruses.^{4–16} While ribavirin has proven to be effective and is generally the standard other antiviral compounds are measured against, it is not without its limitations. First, there are concerns about its side-effects such as hemolytic anemia and potential teratogenicity.^{17,18} Second, it is most effective when administered early in disease; therefore, developing a therapeutic that is better tolerated and effective at later time points in infection a priority. The antiviral T-705 was shown to be effective against a portion of the *Bunyaviridae* family: LACV, Puntó Toro virus (genus *Phlebovirus*) and an attenuated strain of RVFV (MP-12 vaccine strain).¹³ It is a purine nucleoside analog that is thought to be selective for the viral polymerase. While this compound was effective at preventing RVFV infection both in vitro and in vivo, its most promising efficacy is against influenza. This compound has not been tested against any member of the *Nairovirus* or the *Hantavirus* genera, which are some of the most divergent viruses within the *Bunyaviridae* family. Thus the need to identify therapeutics that are effective against all human-affecting members of the *Bunyaviridae* family is of great interest.

A relatively new molecule, FGI-106, was described recently as having antiviral activity against Ebola, dengue,

human immunodeficiency virus, and hepatitis C as well as an attenuated strain of RVFV, the vaccine strain MP-12.¹⁹ Because FGI-106 has shown efficacy against a spectrum of viruses to include an attenuated RVFV strain, we decided to look at its ability to inhibit viral replication among all genera of the *Bunyaviridae* family that are associated with human disease to include a highly pathogenic RVFV strain. Here we show that FGI-106 has antiviral properties *in vitro* against all members of the *Bunyaviridae* family that cause human disease and that it is capable of enhancing survival in a mouse model of RVFV.

Materials and methods

Viral strains

ANDV, strain Chile-9717869, was isolated from the kidneys and lungs of *Oligoryzomys longicaudatus* during an outbreak in Chile and was plaque purified twice as previously described²⁰ before use. HTNV, strain 76-118, was isolated in 1976 from the lungs of *Apodemus agrarius* and propagated in Vero E6 cells before use. CCHFV strain IbAr 10200 was isolated in 1966 from ticks in Nigeria²¹ and was passaged three times in SW13 cells, twice in HepG2 cells, and once in CER cells before use. LACV, strain 97WV-131, was isolated from a human from West Virginia who was stricken with encephalitis in 1997, and was passaged three times in Vero cells before use. RVFV, strain ZH501, was isolated from a febrile human during the 1977 epidemic in Egypt and was passaged twice in fetal rhesus lung cells, once in hamster, and once in BHK cells before use.

Virus yield reduction assays

The effectiveness of FGI-106 was evaluated by virus yield reduction assay using the same cell line of Vero E-6 cells (American Type Culture Collection, Manassas, VA) for analysis of ANDV, HTNV, CCHFV, LACV, and RVFV. The cells were maintained in Modified Eagle's medium (MEM) with 10% fetal bovine serum (FBS), and 1X GlutaMax (Invitrogen, Carlsbad, CA). CCHFV, LACV, and RVFV were evaluated using 90% confluent cells in 6-well plates and the hantaviruses were evaluated in 6- to 7-day-old confluent Vero E-6 cells grown in T150 flasks. Medium was removed from cells, which were infected at a multiplicity of infection (MOI) of 0.1 in 200 μ L of MEM containing 5% FBS, and no antibiotics that contained either 1 μ M or 0.1 μ M. Ribavirin (MP Biomedicals, Solon, OH) was used for comparison purposes as a positive control for the assay at a concentration of 205 μ M (this concentration has proven to be effective in our assay previously). Plates were incubated 1 h at 37°C/5% CO₂

with rocking every 15 min. Medium that contained virus was removed and plates were washed three times with medium or phosphate-buffered saline (PBS; Invitrogen). After washing, medium that contained the drug concentrations above was added and plates were incubated at 37°C/5% CO₂. Supernatant was collected on day 1 postinfection (PI) for CCHFV, LACV, and RVFV, and on day 2 PI for ANDV and HTNV for viral titer determination by plaque assay.

Plaque assays

Hantaviruses

Hantavirus plaque assays were performed as previously described.²⁰ Briefly, supernatant samples were centrifuged to pellet any debris before dilution. Once sample dilutions were made 200 µL was added to each well of a 6-well plate containing 7-day-old Vero E-6 cells. After 1 h adsorption at 37°C/5% CO₂, 3 mL of overlay medium was added to each well, as previously described.¹² Plates were incubated at 37°C/5% CO₂ for 7 days after which they were stained with a 2 mL overlay medium containing 5% FBS and 5% neutral red solution (Invitrogen). Plates were then incubated for an additional 3 days at 37°C or until plaques were visible.

CCHFV

The plaque assay used to determine the CCHFV yield for each concentration of FGI-106 was previously described by Shepherd and colleagues.²² Plaque assays of CCHFV samples used CER cells (USAMRIID), which were maintained in MEM with 10% FBS and 1X GlutaMax. Briefly, CER cells were used once they were 90% confluent in 6-well plates. The medium was removed from each well, and the CER cells were infected with 200 µL of a 10-fold dilution series of the supernatant samples from the yield reduction assay. The plates were incubated for 1 h at 37°C/5% CO₂, then a primary overlay consisting of 1X EBME, 5% FBS, 2% L-glutamine (Hyclone, Logan, UT), 0.5% penicillin-streptomycin (Invitrogen), and 0.8% SeaKem ME agarose (Karlan, Cottonwood, AZ) was added to the monolayer. On day 4 PI, a secondary overlay, which was the same as the primary overlay with the addition of 5% neutral red (Invitrogen), was added to the wells. The plaques were counted on day 5 PI. Visibility of plaques was improved with an additional room temperature incubation overnight.

LACV and RVFV

Plaque assays for LACV and RVFV used 90%–100% confluent Vero cells in 12-well plates. Samples for titration were serially diluted 10-fold and 100 µL was added to each well.

Plates were incubated for 1 h at 37°C with rocking every 15 min. A primary overlay containing 1X EBME, 5% FBS, and 0.5% agarose was added to each well. Plates were incubated at 37°C/5% CO₂ for 3 days followed by a secondary overlay, which was the same as the primary overlay with the addition of 5% neutral red. Plaques were counted on day 4 PI.

Toxicity of FGI-106 *in vitro*

The toxicity of FGI-106 was evaluated in 96-well Vero E-6 cells. The same concentration of compound used for the yield reduction assay was added to the cells and the toxicity was evaluated using Promega's CellTiter-Glo luminescent cell viability assay (Madison, WI) according to the manufacturer's recommendations. Briefly, compounds were added to the plate in triplicate and incubated at 37°C/5% CO₂. After the appropriate incubation period, medium containing test compound was removed and replaced with fresh medium without compound and the CellTiter-Glo substrate. The plates were rocked for 2 min followed by a 10 min incubation at room temperature to allow the signal to stabilize. Plates were read on a luminometer (SpectraMax M5, Molecular Devices, Sunnyvale, CA) at an integration time of 500 ms.

Mouse efficacy studies

Female BALB/c mice were obtained from the National Cancer Institute, Frederick Cancer Research and Development Center (Frederick, MD) and were used at 6–8 weeks. Mice were housed in microisolator cages and were provided water and chow *ad libitum*. FGI-106 was dissolved in dimethylsulfoxide (DMSO) and prepared at the appropriate concentration in PBS. FGI-106 was then administered to mice (n = 10 per group) by intraperitoneal (IP) injection in a total volume of 200 µL. Treatment of mice began 2 h before virus exposure and continued either on alternating days, daily, or twice daily for 10 consecutive days. Mice were challenged subcutaneously with 10, 50, or 100 plaque-forming units (PFU) of RVFV in a total volume of 100 µL. Mice weights and survival was monitored daily (when moribund, observations increased to twice daily).

For one cohort of mice, viral loads were evaluated in tissues and sera from mice on day 3 PI (n = 5 per group). Animals were anesthetized and exsanguinated by cardiac stick and whole blood was analyzed for complete blood counts (CBC) using a hemavet (Drew Scientific, Dallas, TX) and chemistry using the comprehensive diagnostic panel analyzed on a Vetscan (Abaxis, Union City, CA). The liver,

spleen, inguinal lymph node, brain, heart, and kidney were collected, weighed, and homogenized in MEM containing 5% FBS and gentamicin. Tissues were homogenized using the Qiagen Mixer Mill 300 (Retsch, Newtown, PA) then centrifuged at 10,000 rpm for 10 min and the supernatant stored at -70°C until further evaluation by standard plaque assay to determine titers. Research at USAMRIID was performed in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the guide for the Care and Use of Laboratory Animals, National Research Council, 1996. USAMRIID is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Pathology

Tissues were fixed in 10% neutral-buffered formalin for a minimum of 21 days, then removed from biocontainment and processed for routine histopathology and stained by hematoxylin and eosin (HE). Duplicate sections were stained by immunohistochemistry for RVFV antigen using a commercially available immunoperoxidase method (EnVision System, DAKO Corp., Carpinteria, CA). Sections were deparaffinized and pretreated with proteinase K for 6 minutes prior to incubation with the primary antibody (R1547 - rabbit polyclonal anti RVFV), diluted 1:800 and incubated at room temperature for 30 min, then followed with a peroxidase-conjugated polymer-based secondary antibody. The severity of liver lesions and the amount of viral antigen was assessed by a pathologist. These parameters were individually characterized on a subjective scale as follows: 1+, minimal; 2+, mild; 3+, moderate; 4+, extensive. In addition, RVFV antigen in the liver was quantitated by digital analysis, using an Automated Cellular Imaging System (ACIS; Dako, Carpinteria, CA). The ACIS image analysis software calculates a percentage score by evaluating the intensity and percentage of positive area (brown color) and quantifies that over the total amount of positive and negative (blue hematoxylin staining) area.

Statistics

SAS version 9.1.3 (SAS Institute Inc., Cary, NC) was used to determine differences in viral titers, clinical chemistry, hematology, survival rate, survival curves, and time-to-death between treatment groups for efficacy studies using Fisher's exact tests with stepdown Bonferroni correction, t -test with stepdown Bonferroni correction, and Kaplan–Meier survival analysis with log-rank comparison of survival curves between groups.

Results

Cell-based inhibition of bunyaviruses by FGI-106

The need to improve treatment for patients infected with bunyaviruses led us to evaluate a series of experimental compounds for their abilities to inhibit multiple types of bunyaviruses. Our initial studies focused on two different hantaviruses using cell-based assays. These particular viruses were selected because they encompass geographically distinct pathogens from South America (ANDV) and Asia (HTNV). Vero E-6 cells were infected with virus in the presence or absence of FGI-106, which was added following virus absorption to cells. Cell-based assays revealed that treatment with FGI-106 caused a significant reduction of virus for both HTNV and ANDV (Figures 1A and 1B, Table 1). Plaque assays indicated that on day 2 PI, FGI-106 at the $1\text{ }\mu\text{M}$ concentration reduced viral titers by at least $1\text{ log}_{10}\text{ PFU/mL}$ relative to matched controls, which was determined to be significant ($P \leq 0.0001$) for both HTNV and ANDV. The reduction in virus occurred in a dose-dependent manner as no reduction was observed using $0.1\text{ }\mu\text{M}$ of compound. There was no toxicity associated with FGI-106 at concentrations of $1\text{ }\mu\text{M}$ or less using an assay that measures ATP as a criterion for metabolically active cells (data not shown). The ribavirin control at $205\text{ }\mu\text{M}$ (this concentration proved effective in previous analysis and was not toxic to cells) proved more effective than FGI-106 and reduced viral titers by 100-fold more. However, when the concentration of ribavirin was matched to that of FGI-106 ($1\text{ }\mu\text{M}$), no reduction in viral titers occurred (data not shown).

Based on the promising findings with ANDV and HTNV, we expanded our investigation to ask if FGI-106 might also have application against additional bunyaviruses. Therefore, we evaluated the compound against CCHFV, a member of the *Nairovirus* genus and against LACV, a member of the *Orthobunyavirus* genus. A significant reduction in virus was observed for CCHFV as well as LACV evaluated against the $1\text{ }\mu\text{M}$ concentration of compound (Figures 1C–1D, Table 1). A $1.2\text{ log}_{10}\text{ PFU/mL}$ reduction in viral titer was observed on day 1 for CCHFV and a $3.0\text{ log}_{10}\text{ PFU/mL}$ reduction for LACV, which was determined to be significant ($P \geq 0.0001$ and $P = 0.0003$ for CCHFV and LACV, respectively). Ribavirin controls ($205\text{ }\mu\text{M}$) proved to be similarly effective as that of FGI-106 (data not shown). Surprisingly, the viral titer was higher for CCHFV evaluated with $0.1\text{ }\mu\text{M}$ of FGI-106 when compared to the no compound control, but this effect is occasionally observed with other compounds evaluated

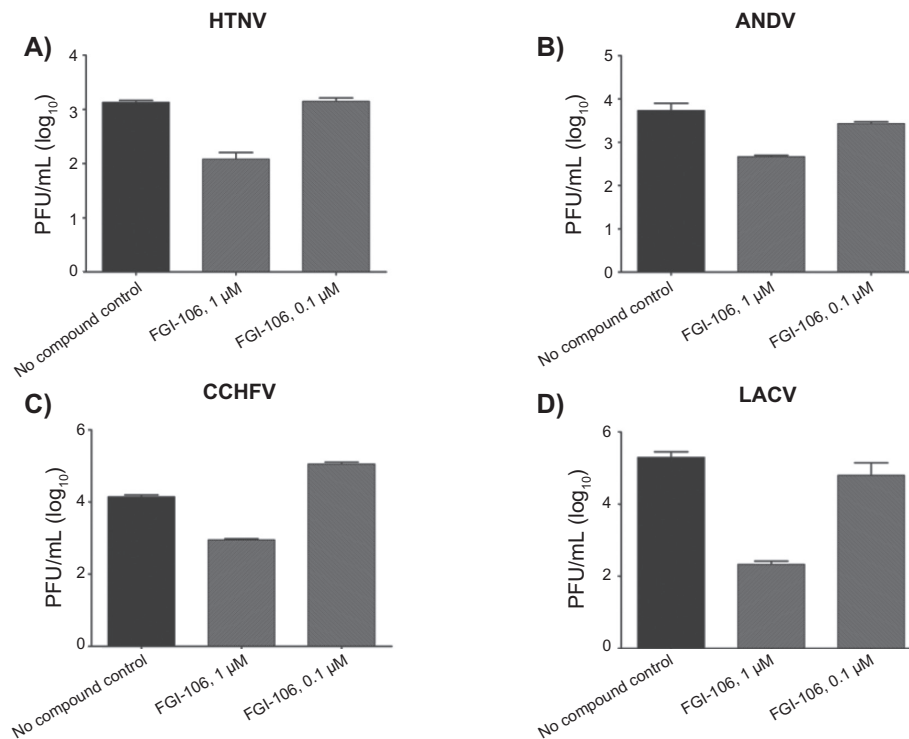


Figure 1 Virus yield reduction assay results for FGI-106 (1 µM or 0.1 µM) evaluated against **A)** HTNV, **B)** ANDV, **C)** CCHFV, and **D)** LACV. Viral titers were determined on day 2 PI for HTNV and ANDV and on day 1 PI for CCHFV and LACV. Error bars represent standard deviations.

Abbreviations: HTNV, Hantaan virus; ANDV, Andes virus; CCHFV, Crimean-Congo hemorrhagic fever virus; LACV, La Crosse virus; RVFV, Rift Valley fever virus; PFU, plaque-forming units.

in our assay as well. It seems that some compounds at certain concentrations can actually cause an enhancement in the replication of the virus for reasons that are unknown when the mechanism of action of the compound is not fully understood.

We then expanded our evaluation to RVFV, a viral hemorrhagic fever in the *Phlebovirus* genus. Similar to the results observed with the other bunyaviruses, FGI-106 treatment decreased RVFV titers in a dose-dependent manner (Figure 2). Antiviral activity at 1 µM was sufficient to cause a significant (3.2 log₁₀ PFU/mL) reduction in RVFV on day 1

PI (Table 1; $P = 0.0006$). Indeed, FGI-106 was more effective against RVFV relative to any other bunyaviruses evaluated in our assay and was 10-fold more effective than the ribavirin control (205 µM concentration; data not shown). A thorough assessment of RVFV release yielded an estimated effective concentration at 90% (EC_{90}) value of 7.4 nM.

Safety and pharmacokinetic analyses of FGI-106 *in vivo*

The promising RVFV inhibitory activity of FGI-106 in cell-based assays led us to ask if the compound might also be effective *in vivo*. The pharmacokinetics and bioavailability of the molecule *in vivo* was published previously.¹⁹ These studies were completed using uninfected mice to investigate the pharmacokinetics and organ distribution of FGI-106 to relevant target organs. Specifically, viral hemorrhagic fevers are often characterized by damage to the liver, kidneys, and spleen. In addition, the drug concentration in the lungs was assessed based on the role of bunyaviruses in HPS. For these pharmacokinetic studies, groups of three mice were given a single dose (3 mg/kg) of FGI-106, administered intravenously, and blood samples

Table 1 Yield reduction in PFU/mL (log₁₀) compared to no compound virus control

Virus	FGI-106, 1 µM	FGI-106, 0.1 µM
HTNV	1.0	0.0
ANDV	1.0	0.3
CCHFV	1.2	-0.9
LACV	3.0	0.5
RVFV	3.2	0.2

Abbreviations: HTNV, Hantaan virus; ANDV, Andes virus; CCHFV, Crimean-Congo hemorrhagic fever virus; LACV, La Crosse virus; RVFV, Rift Valley fever virus; PFU, plaque-forming units.

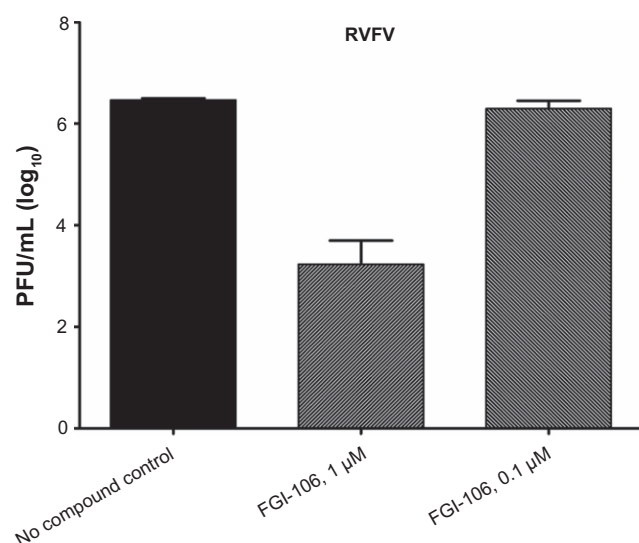


Figure 2 Virus yield reduction assay results for FGI-106 (1 µM or 0.1 µM) evaluated against RVFV. Viral titers were determined on day 1 PI. Error bars represent standard deviations.

Abbreviations: RVFV, Rift Valley fever virus; PFU, plaque-forming units.

were collected over the following 6 h. These studies revealed a peak FGI-106 plasma concentration of 286 ng/mL 5 min after dosing and the plasma levels decreased thereafter with an estimated half life of approximately 15 min (Figure 4A in¹⁹). Organs were harvested 6 h after mice received a single 3 mg/kg dose, revealing accumulation of

10 to 40 µg of FGI-106 per g in organs of interest (lungs, liver, kidneys, spleen; Figure 4B in¹⁹). To place these findings in perspective of the results of cell-based studies, the EC₉₀ value determined with Vero cells translates to 4.4×10^{-3} µg/g. These findings thus predicted treatment levels of 3 mg/kg could be sufficient to impact RVFV infection *in vivo*.

To investigate the potential efficacy of FGI-106 *in vivo*, a lethal model of RVFV was implemented in which mice were challenged with 100 PFU of virus. For our initial investigation, groups of 10 mice were treated with 5 mg/kg of FGI-106 (or a matched vehicle control, DMSO) 2 h before exposure (100 PFU of RVFV) and treatment continued every other day for the next 10 days. The median survival of matched control mice receiving DMSO was 4 days PI (mean time to death 4.5 days) while the median survival of FGI-106-treated mice was 7 days PI (mean time to death 7.5 days). Thus, treatment with FGI-106 extended the survival of RVFV infected mice by 3 days (Figure 3, Table 2), which was found to be significant in regards to time to death ($P = 0.0115$) and the survival curve ($P = 0.0225$).

The prolonged survival of FGI-106-treated mice led us to investigate further the impact of drug treatment on RVFV pathogenesis. Mice were infected with RVFV and euthanized three days PI, which is the point at which the majority of

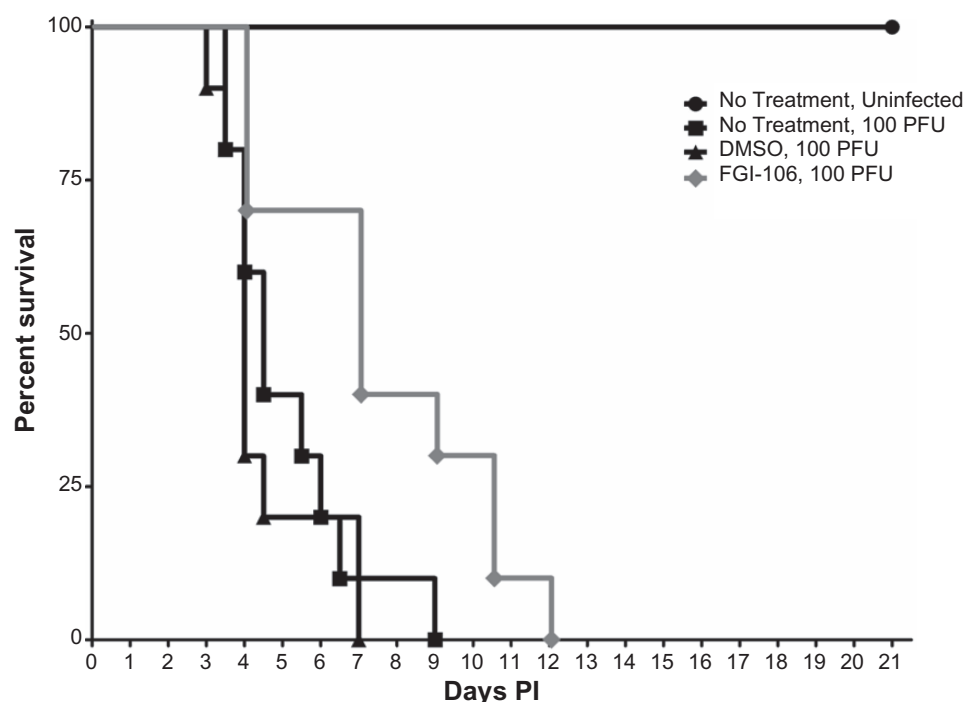


Figure 3 Percent survival of FGI-106-treated animals exposed to 100 PFU of RVFV. Mice were treated with 5 mg/kg of FGI-106 every other day for 10 consecutive days (beginning 2 h before virus exposure).

Abbreviations: PFU, plaque-forming units; RVFV, Rift Valley fever virus; PI, post-infection.

Table 2 Descriptive statistics of FGI-106-treated (5 mg/kg every other day) and control mice

Group	Total	Dead		Alive		Days to death		Survival time		
	N	N	%	N	%	Mean	SD	Median	Mean	SEM
DMSO	10	10	100	0	0	4.5	1.4	4.0	4.5	0.4
FGI-106	10	10	100	0	0	7.5	2.9	7.0	7.5	0.9
Control	10	10	100	0	0	5.1	1.7	4.5	5.1	0.5

Abbreviations: DMSO, dimethylsulfoxide; SEM, standard error of mean; SD, standard deviation.

mice succumb to the infection. Due to the shorter duration of the study, we also modified the dosing and frequency to minimize the potential for drug-induced toxicity (2 mg/kg once a day). Interestingly, the viral load in the sera and liver did not significantly differ when comparing FGI-106-treated mice and DMSO controls (Figure 4A). Likewise, the viral load in the spleen, inguinal lymph node, heart, kidneys, and brain of FGI-106 treated mice compared to DMSO controls was not significantly different (data not shown). In contrast to viral load, FGI-106 did appear to protect target organs from RVFV-mediated damage. Specifically, RVFV damage to the liver is the major cause of its pathogenicity. Thus, we utilized elaboration of liver enzymes as markers of liver distress and found significantly higher evidence of alanine aminotransferase (Figure 4B; $P = 0.0002$) and alkaline phosphatase (Figure 4C; $P \leq 0.0001$) in virus-infected, DMSO-treated animals as compared to infected mice treated with FGI-106. Additionally, we observed a significant increase in the total bilirubin (Figure 4D; $P = 0.0019$) and a significant decrease in the glucose (Figure 4E; $P = 0.0102$) and calcium (Figure 4F; $P = 0.0004$) levels of infected FGI-106-treated mice compared to infected DMSO controls. These results suggest that the efficacy of FGI-106 may involve activities that prevent RVFV damage to the liver. Hematological analysis of infected FGI-106-treated mice compared to infected DMSO controls revealed significantly higher levels of basophils (Figure 5A; $P = 0.0200$) and lower levels of red blood cells (Figure 5B; $P = 0.0110$), hemoglobin (Figure 5C; $P = 0.0226$), and hematocrit (Figure 5D; $P = 0.0147$) levels.

The livers from FGI-106-treated mice vs untreated DMSO controls were further evaluated for differences in histology and immunohistochemical staining of RVFV antigen. The amount of viral antigen in the livers of DMSO-treated mice was extensive (Figure 6A) and similar to the amounts seen in the livers of untreated mice infected with RVFV and sacrificed at day 3 PI in a previous study (Smith, unpublished observations). Virus antigen was present primarily in hepatocytes (mean score of 3.4; range of 3.0–3.5) and 52% of cells were determined to be IHC positive by the ACIS.

Lesions in these mice were also similar to those seen in the previous study at day 3 PI. The primary change in the livers of DMSO-treated mice infected with RVFV was the presence of multifocal to coalescing hepatocyte apoptosis (Figure 6B) judged to be moderate to marked in severity (mean score of 3.6; range of 3.0–4.0). Infiltrates of a few viable and degenerate neutrophils and occasional lymphocytes were observed in four of the five cases in this group.

The amounts of viral antigen in the livers of FGI-106-treated mice (Figure 6C) were typically less than in DMSO-treated mice. Virus antigen was present primarily in hepatocytes of mice in the treated group as well (mean score of 2.5; range of 1.5–3.5) and 26% of cells were determined to be IHC positive by the ACIS. Lesions in the FGI-106-treated mice were milder (Figure 6D; mean score of 1.8; range of 1.0–2.5) than those in the untreated control group, though qualitatively similar. Individual (arrowheads) or small foci of apoptotic hepatocytes were typical of these mice and inflammatory infiltrates similar to those in the untreated control mice were evident in all five cases in this group.

The prolonged survival and improved liver function in FGI-106-treated animals that had been challenged with a relatively robust infection with RVFV led us to ask if we might improve FGI-106 efficacy by decreasing the stringency of the initial viral challenge. For this, we decreased the challenge dose of RVFV to 10 PFU per animal for one cohort and assessed subsequent survival. Assessment of overall survival revealed that 90%–100% of matched control mice succumbed to infection within 4–5 days (median survival of 5.25 days and mean time to death of 5.4 days PI; Figure 7, Table 3). In contrast, the FGI-106-treated animals survived longer (median survival of 7.5 days and mean time to death of 5.8 days PI) and approximately 40% of the animals were able to survive infection altogether; however, the comparison of survival and times to death of treated animals vs untreated was not found to be statistically significant. To verify that all surviving subjects had developed a protective immune response during the initial challenge, all survivors were back-challenged subcutaneously with a lethal dose of RVFV (100 PFU) 30 days PI and 50% (2/4) mice survived, thus

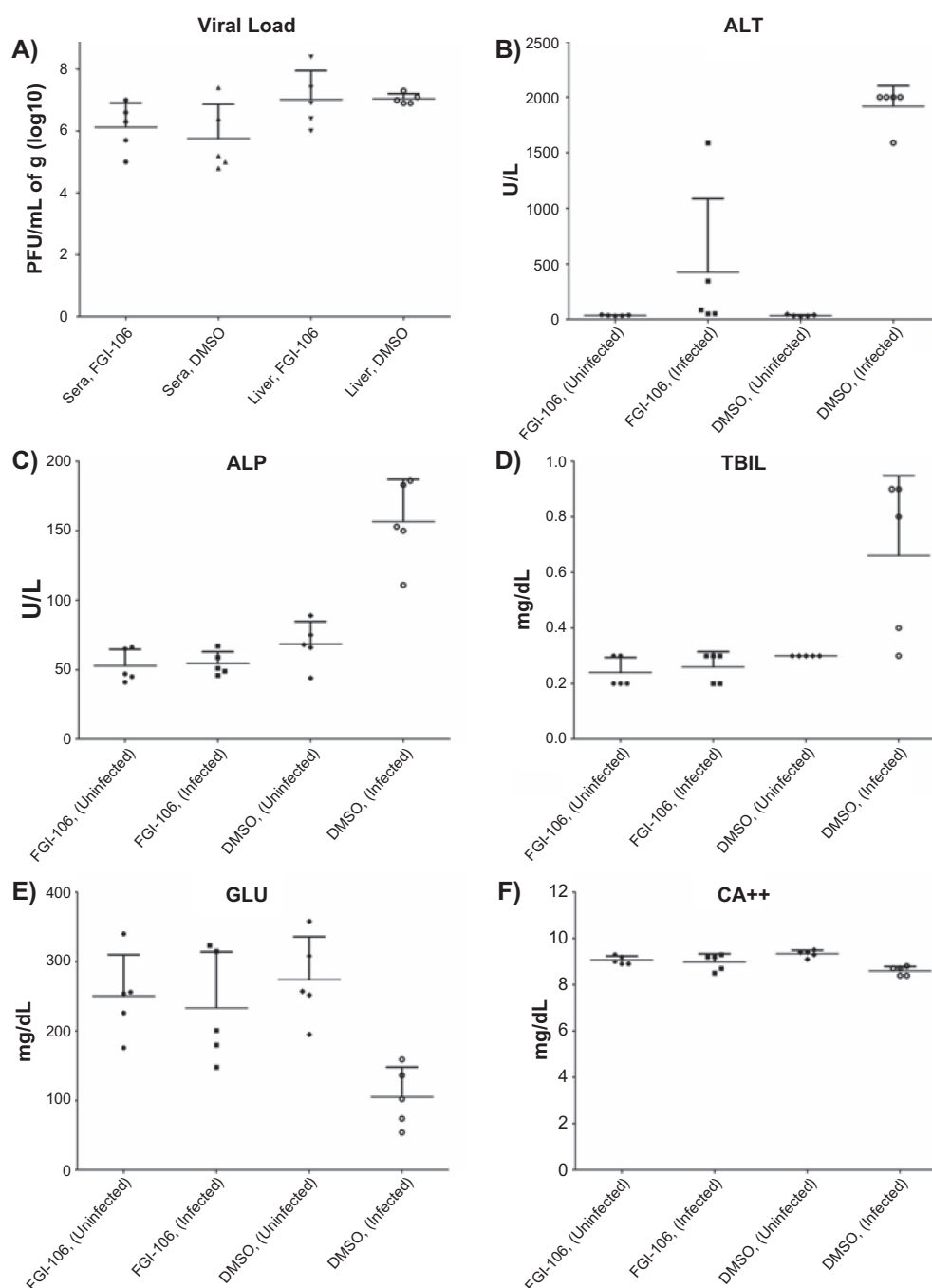


Figure 4 Analysis of the **A)** viral load, **B)** liver enzyme alanine aminotransferase (ALT), **C)** liver enzyme alkaline phosphatase (ALP), **D)** total bilirubin (TBIL), **E)** glucose (GLU), **F)** calcium (CA++) in FGI-106 or DMSO control-treated mice on day 3 PI. The viral titer was determined in the sera and livers of DMSO controls compared to FGI-106-treated mice ($n = 5/\text{group}$). The ALT, ALP, TBIL, GLU, and CA++ levels were determined in the whole blood of DMSO controls and FGI-106-treated mice ($n = 5/\text{group}$). Error bars represent standard deviations.

Abbreviations: DMSO, dimethylsulfoxide; PFU, plaque-forming units.

confirming the presence of some level of neutralizing antibody. Similar to the previous study, mice infected with 100 PFU and treated with FGI-106 showed a significant ($P = 0.0219$) improvement in the mean time to death when compared to DMSO controls (7.3 vs 3.7 days PI, respectively). Although improved relative to vehicle-treated controls, we also could

not exclude that direct toxicity of the compound might have contributed to some of the mortality in the FGI-106-treated animals and that this might have under-represented the antiviral activity of FGI-106 in vivo. Altogether, these findings suggest that FGI-106 can contribute to protection from an otherwise lethal infection with RVFV.

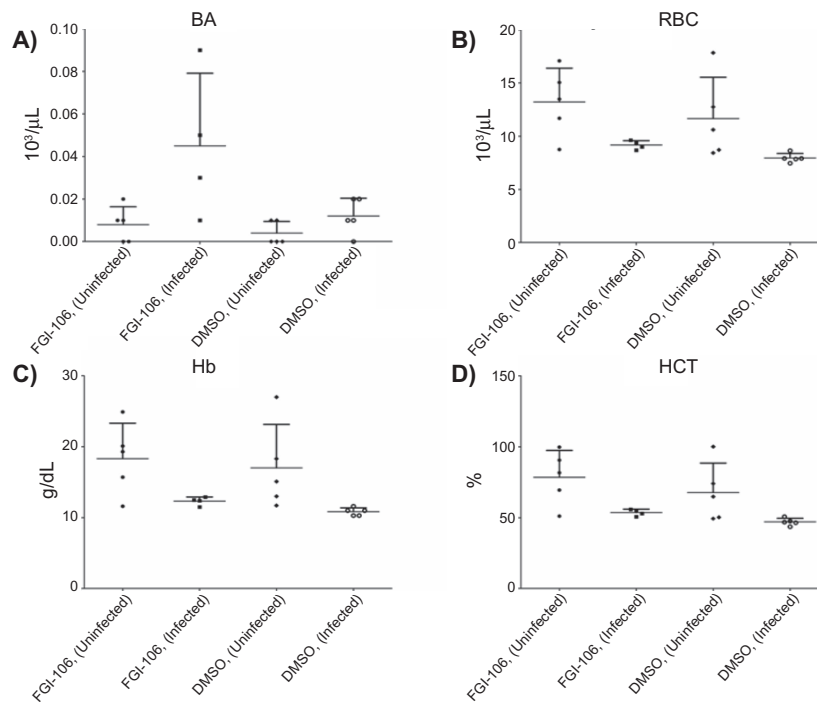


Figure 5 Hematological analysis of the **A)** basophils (BA), **B)** red blood cells (RBC), **C)** hemoglobin (Hb), and **D)** hematocrit (HCT) levels in FGI-106 or DMSO control-treated mice on day 3 PI. Error bars represent standard deviations.

Abbreviation: DMSO, dimethylsulfoxide.

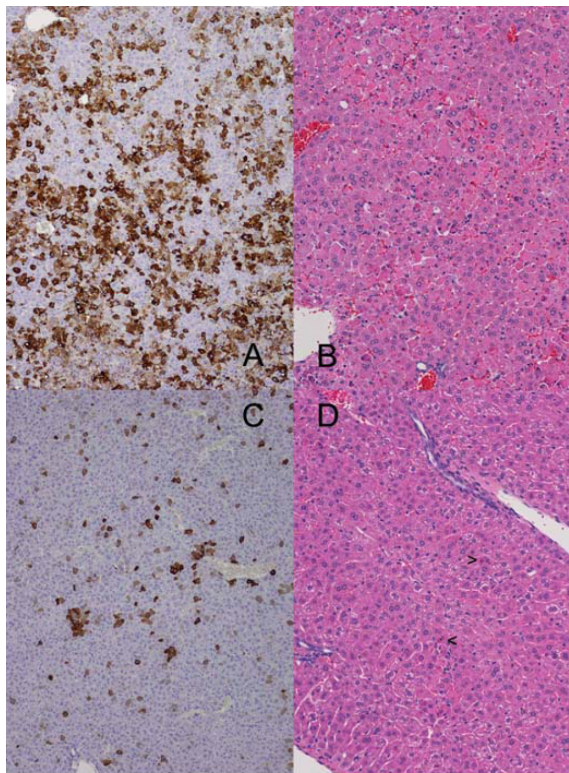


Figure 6 Analysis of the viral antigen (**A** and **C**) and histology (**B** and **D**) in **A–B)** FGI-106-treated animals and **C–D)** DMSO-treated control animals on day 3 PI ($n = 5/\text{group}$).

Abbreviation: DMSO, dimethylsulfoxide.

Discussion

The major finding of our present work is a demonstration that FGI-106 can inhibit multiple genera of bunyaviruses, including intractable viruses such as hantaviruses, CCHFV, and RVFV. This indicates that, for the first time, the development of a broad-spectrum bunyavirus therapeutic is possible. We observed a range of inhibition using cell-based assays of at least $1 \log_{10}$ PFU/mL for ANDV and HTNV to as high as $3.2 \log_{10}$ PFU/mL for RVFV. The pharmacokinetic and organ distribution profile of FGI-106 *in vivo* was previously shown to be sufficient to provide efficacious levels of FGI-106 to organs that are relevant to infection with bunyaviruses (liver, lungs, spleen, and kidneys;¹⁹). Consistent with this finding, FGI-106 treatment of animals infected with RVFV was sufficient to delay the onset of disease and protect almost half of individuals from an otherwise lethal infection.

These results are encouraging as RVFV infection in humans is not 100% lethal as in the mouse model of RVFV. Mice are highly susceptible to infection with RVFV which leads to the development of fulminant hepatitis and a late-developing encephalitis,²³ which is similar to the manifestations observed in human disease, but to a lesser degree of severity in most cases. Interestingly, our results suggest that viral load was not a particularly good predictor of the efficacy

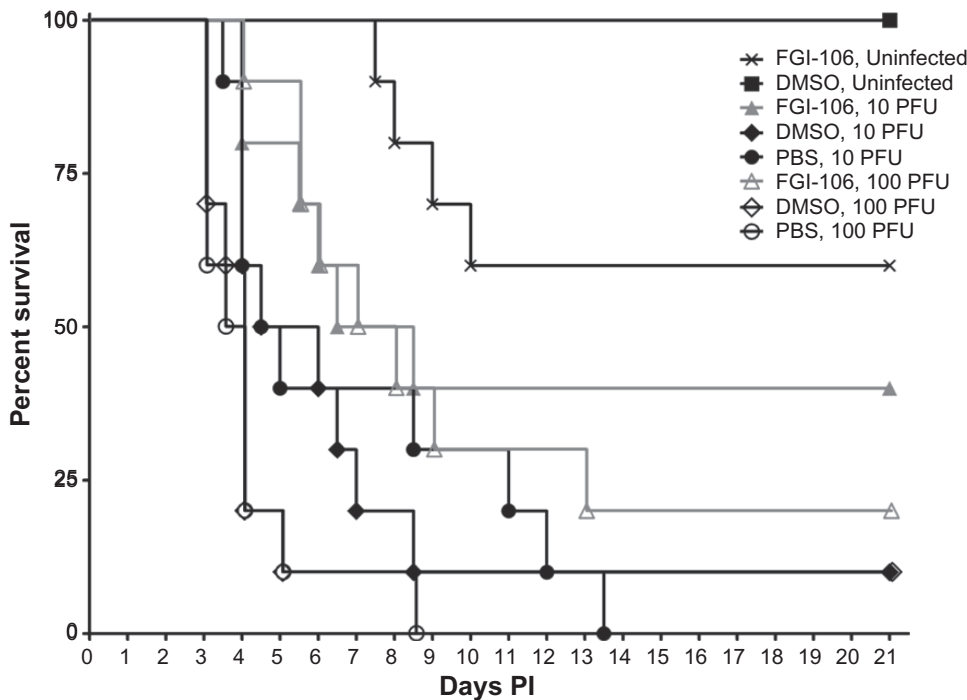


Figure 7 Percent survival of FGI-106-treated animals exposed to 10 or 100 PFU of RVFV. Mice were treated with 2 mg/kg of FGI-106 twice a day for 10 consecutive days (beginning 2 h before virus exposure).
Abbreviations: PFU, plaque-forming units; RVFV, Rift Valley fever virus; PI, post-infection.

of FGI-106, but that the preservation of the host organ function was, as suggested by our analysis of liver enzymes and hematological changes in treated versus untreated mice. The liver, which is the predominant target of RVFV, was protected to some degree as shown by our analysis of the histology and detection of viral antigen by IHC in FGI-106 treated vs untreated control mice. Our *in vitro* and *in vivo* results collectively suggest that FGI-106 activity may not entirely be due to antiviral effects, but more of a therapeutic effect.

FGI-106 was originally identified using a screen of small-molecule inhibitors of Ebola virus,¹⁹ which is a hemorrhagic fever virus in the family *Filoviridae*. These studies demonstrated efficacy against Ebola virus as measured in

cell-based studies and *in vivo* in a mouse model of infection. Studies by Aman et al also evaluated the effectiveness of FGI-106 using an attenuated strain of RVFV (MP-12), which they found to be mildly effective. However, we evaluated a highly pathogenic strain of RVFV using a different cell-based assay thus making a direct comparison of our results inappropriate. Our present findings are unique, in part, because we expanded the efficacy of FGI-106 to additional bunyaviruses, a genetically and functionally distinct family of hemorrhagic fever, pulmonary, and encephalitic viruses. This finding is interesting as the utilization of FGI-106, or derivatives thereof, might provide a means to treat multiple and different types of hemorrhagic fever viruses with a single therapeutic.

Table 3 Descriptive statistics of FGI-106-treated (2 mg/kg daily) and control mice

PFU	Total		Dead		Alive		Days to death		Survival time		
	Group	N	N	%	N	%	Mean	SD	Median	Mean	SEM
10	DMSO	10	9	90	1	10	5.4	1.7	5.25	7.0	1.6
10	FGI-106	10	6	60	4	40	5.8	1.7	7.50	11.9	2.6
10	PBS	10	10	100	0	0	7.0	3.9	4.75	7.0	1.2
100	DMSO	10	9	90	1	10	3.7	0.7	4.00	5.5	1.8
100	FGI-106	10	8	80	2	20	7.3	2.8	7.50	10.0	2.0
100	PBS	10	10	100	0	0	4.1	1.7	3.75	4.1	0.5

Abbreviations: DMSO, dimethylsulfoxide; PBS, phosphate-buffered saline; SD, standard deviations; SEM, standard error of mean.

At present, the mechanistic basis of FGI-106 antiviral activity remains unknown. We observed varying degrees of the effectiveness of FGI-106 for the different bunyaviruses analyzed. In the context of our present findings and previous work demonstrating antiviral activity against genetically distinct viruses (eg, Ebola), we postulate that the molecule targets a host pathway that is shared by different viral types. An ongoing investigation seeks to identify such a pathway. Future studies will evaluate additional analogues that may be similarly effective for all the bunyaviruses. The apparent toxicity of the FGI-106 itself might have under-estimated the magnitude of antiviral efficacy. Thus, future studies are also identifying analogues and variants of FGI-106 that might provide a larger margin of safety while maintaining or increasing the robust, broad-spectrum antiviral activity. These analogues should also provide insight into the mechanism of action of FGI-106. Interestingly, it appears that infected mice treated with FGI-106 experienced an increase in basophil levels that was not due to treatment with the drug alone. This result, along with the other chemistry and hematological changes observed, offers an interesting avenue for further research into the mechanism of action of FGI-106 in RVFV-infected animals.

Our present findings suggest an opportunity for future treatment of *Bunyavirus* infections for which can have significant mortality associated with disease. For example, the case-fatality rate for HTNV ranges from 5–15% and the fatality rate for ANDV range from 30%–50%.²⁴ The mortality rate associated with RVF in humans ranges from 1%–3% and has been reported to be as high as 14% during a relatively recent epidemic in Saudi Arabia where a high incidence of neurological manifestations (17.1%) was reported in infected persons.^{25,26} Infection with CCHFV leads to mortality rates as high as 10%–50%.²⁴ Currently, no antivirals have been approved to treat *Bunyavirus* infection in humans; therefore, supportive care is the primary source of treatment and intensive care is often required. The use of ribavirin has proven effective against RVFV in rodent and non-human primate models,⁷ but has not been thoroughly evaluated to treat RVFV infection in humans. During the 2000 Saudi Arabia outbreak, a small clinical trial evaluating the effectiveness of ribavirin to treat severe RVFV infection suggested an increase in the incidence of encephalitis in ribavirin-treated cases; however, these results are inconclusive (P. Rollin, presented at the Treatment of Viral Hemorrhagic Fever Workshop, Bethesda, MD, February 24 to 27, 2007). Ribavirin has proven effective against CCHFV in cell culture, mice,^{10,11} and several

human cases²⁷ when administered early during the disease course. However, issues with the use of ribavirin leading to hemolytic anemia and potential teratogenicity still remain a major concern.^{17,18} In the case of hantavirus infection, there is evidence that ribavirin might be useful for treatment of HFRS, but ribavirin does not appear to be effective for HPS. This latter finding is notable in light of our preliminary evidence that FGI-106 can become enriched within the lung at levels sufficient to block hantavirus infection (as indicated by cell-based assays). Thus, future investigation should determine whether FGI-106 might provide utility for the treatment of hantavirus infections, including HPS.

In conclusion, the development of novel therapeutics to treat *Bunyavirus* infection is of particular interest. The bunyaviruses discussed here are characterized as category A (RVFV, hantaviruses), B (LACV), or C (CCHFV) agents by the US NIAID, making therapeutic development for these viruses a top priority. Therefore, further development of broad-spectrum therapeutics, such as FGI-106, is paramount.

Acknowledgments

We thank Ashley Keeney, Calli Lear, and Hanwen Mao for technical assistance, Keith Steele and Maureen Kennedy for pathology support, and Sarah Norris for statistical analysis support. We would like to thank Dr Jay W. Hooper for the hantaviruses used in this experiment and the CDC for kindly providing the LACV. This work was supported by the Defense Threat Reduction Agency (DTRA) and Monica Ogg is supported by a National Research Council fellowship funded by a grant awarded by the Military Infectious Disease Research Program, U.S. Army Medical and Material Command, project no. T0041_09_RD and NIAID grant award 1R21AI064499-01. The views of the authors do not necessarily reflect the position of the Department of Defense or the Department of the Army.

References

1. Fields BN, Knipe DM, Griffin DE. *Fields' Virology*. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams and Wilkins; 2007.
2. Meegan JM, Bailey CL. Rift Valley Fever. In: Monath TP, editor. *The Arboviruses: Epidemiology and Ecology*. Boca Raton, FL: CRC Press; 1989; IV:51–76.
3. Flick R, Bouloy M. Rift Valley fever virus. *Curr Mol Med*. 2005;5(8):827–834.
4. Canonico PG, Kende M, Luscri BJ, Huggins JW. In-vivo activity of antivirals against exotic RNA viral infections. *J Antimicrob Chemother*. 1984;14(Suppl A):27–41.
5. Huggins JW, Robins RK, Canonico PG. Synergistic antiviral effects of ribavirin and the C-nucleoside analogs tiazofurin and selenazofurin against togaviruses, bunyaviruses, and arenaviruses. *Antimicrob Agents Chemother*. 1984;26(4):476–480.

6. Huggins JW, Kim GR, Brand OM, McKee KT Jr. Ribavirin therapy for Hantaan virus infection in suckling mice. *J Infect Dis.* 1986;153(3):489–497.
7. Peters CJ, Reynolds JA, Slone TW, Jones DE, Stephen EL. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. *Antiviral Res.* 1986;6(5):285–297.
8. Cassidy LF, Patterson JL. Mechanism of La Crosse virus inhibition by ribavirin. *Antimicrob Agents Chemother.* 1989;33(11):2009–2011.
9. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev Infect Dis.* 1989;11 (Suppl 4): S750–S761.
10. Watts DM, Ussery MA, Nash D, Peters CJ. Inhibition of Crimean-Congo hemorrhagic fever viral infectivity yields in vitro by ribavirin. *Am J Trop Med Hyg.* 1989;41(5):581–585.
11. Tignor GH, Hanham CA. Ribavirin efficacy in an in vivo model of Crimean-Congo hemorrhagic fever virus (CCHF) infection. *Antiviral Res.* 1993;22(4):309–325.
12. Chung DH, Sun Y, Parker WB, et al. Ribavirin reveals a lethal threshold of allowable mutation frequency for Hantaan virus. *J Virol.* 2007;81(21):11722–11729.
13. Gowen BB, Wong MH, Jung KH, et al. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. *Antimicrob Agents Chemother.* 2007;51(9):3168–3176.
14. Chung DH, Kumarapperuma SC, Sun Y, et al. Synthesis of 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole and its in vitro and in vivo efficacy against Hantavirus. *Antiviral Res.* 2008;79(1):19–27.
15. Jonsson CB, Hooper J, Mertz G. Treatment of hantavirus pulmonary syndrome. *Antiviral Res.* 2008;78(1):162–169.
16. Rusnak JM, Byrne WR, Chung KN, et al. Experience with intravenous ribavirin in the treatment of hemorrhagic fever with renal syndrome in Korea. *Antiviral Res.* 2009;81(1):68–76.
17. Snell NJ. Ribavirin – current status of a broad spectrum antiviral agent. *Expert Opin Pharmacother.* 2001;2(8):1317–1324.
18. Borio L, Inglesby T, Peters CJ, et al; Working Group on Civilian Bio-defense. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA.* 2002;287(18):2391–2405.
19. Aman MJ, Kinch MS, Warfield K, et al. Development of a broad-spectrum antiviral with activity against Ebola virus. *Antiviral Res.* 2009;83(3):245–251.
20. Hooper JW, Larsen T, Custer DM, Schmaljohn CS. A lethal disease model for hantavirus pulmonary syndrome. *Virology.* 2001;289(1):6–14.
21. Causey OR, Kemp GE, Madbouly MH, David-West TS. Congo virus from domestic livestock, African hedgehog, and arthropods in Nigeria. *Am J Trop Med Hyg.* 1970;19(5):846–850.
22. Shepherd AJ, Swanepoel R, Leman PA, Shepherd SP. Comparison of methods for isolation and titration of Crimean-Congo hemorrhagic fever virus. *J Clin Microbiol.* 1986;24(4):654–656.
23. Peters CJ, Linthicum KJ. Rift Valley Fever. In: Beran GW, Steele JH, editors. *Handbook of Zoonoses, Section B. Viral.* Boca Raton, FL: CRC Press; 1994:125–138.
24. Soldan SS, Gonzalez-Scarano F. Emerging infectious diseases: the Bunyaviridae. *J Neurovirol.* 2005;11(5):412–423.
25. Balkhy HH, Memish ZA. Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. *Int J Antimicrob Agents.* 2003;21(2):153–157.
26. Madani TA, Al-Mazrou YY, Al-Jeffri MH, et al. Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. *Clin Infect Dis.* 2003;37(8):1084–1092.
27. Fisher-Hoch SP, Khan JA, Rehman S, et al. Crimean Congo-haemorrhagic fever treated with oral ribavirin. *Lancet.* 1995;346(8973):472–475.

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