Antiviral Research 83 (2009) 245–251

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Development of a broad-spectrum antiviral with activity against Ebola virus

M. Javad Aman[a,1,2], Michael S. Kinch[b,*,1], Kelly Warfield[a,2], Travis Warren[a], Abdul Yunus[b], Sven Enterlein[c], Eric Stavale[c], Peifang Wang[d], Shaojing Chang[b], Qingsong Tang[b], Kevin Porter[d], Michael Goldblatt[b], Sina Bavari[a]

[a] United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD 21702, USA
[b] Functional Genetics, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878, USA
[c] Integrated BioTherapeutics, Germantown, MD 20876, USA
[d] Naval Medical Research Center, Silver Spring, MD 20910, USA

ARTICLE INFO

Article history:
Received 17 March 2009
Received in revised form 20 May 2009
Accepted 3 June 2009

Keywords:
Ebola virus
Antiviral
Dengue Fever

ABSTRACT

We report herein the identification of a small molecule therapeutic, FGI-106, which displays potent and broad-spectrum inhibition of lethal viral hemorrhagic fevers pathogens, including Ebola, Rift Valley and Dengue Fever viruses, in cell-based assays. Using mouse models of Ebola virus, we further demonstrate that FGI-106 can protect animals from an otherwise lethal infection when used either in a prophylactic or therapeutic setting. A single treatment, administered 1 day after infection, is sufficient to protect animals from lethal Ebola virus challenge. Cell-based assays also identified inhibitory activity against divergent virus families, which supports a hypothesis that FGI-106 interferes with a common pathway utilized by different viruses. These findings suggest FGI-106 may provide an opportunity for targeting viral diseases.

1. Introduction

Highly pathogenic members belonging to families of Filo-, Flavi-, Arena- and Bunya-viridae are implicated in causing Viral Hemorrhagic Fevers (VHFs) (Burnett et al., 2005; Hensley et al., 2005; Khan et al., 2008; Marty et al., 2006). These viruses cause a deadly infection in humans with high case fatality rates (Zampieri et al., 2007). Nonetheless, natural outbreaks of these agents are often sporadic and restricted to underdeveloped or isolated pockets of the population. A prominent exception is Dengue Fever, which is widespread throughout tropical regions, with tens of millions of cases occurring annually (Barreto and Teixeira, 2008). The fact that these viruses are generally prevalent in developing countries has unfortunately posed a financial disincentive for many large pharmaceutical companies to pursue an effective antiviral therapy for VHFs. The need for specialized BSL4 facilities with trained personnel has further restricted research to a few high-security sites worldwide. As a result, there are no FDA approved antivirals for Ebola and many other VHFs except supportive therapy (De Clercq, 2008). Although ribavirin is approved for treatment of other pathogens, this medication has been used to treat several types of VHF, although there remains a need to replace this option with a more potent and less toxic medication (Bray, 2008).

A lack of treatment options is an alarming situation for public health officials around the world in light of the potential for intentional or accidental releases of such agents and thereby poses national security and economic threats beyond the normal ranges of these viruses. The need for an effective therapeutic or vaccine for VHF was painfully reinforced by a deadly reemergence of Dengue Fever that assumed epidemic proportions in Brazil and caused a national emergency.

In this report, we present the discovery of a novel broad-spectrum small molecule inhibitor, FGI-106, with inhibitory activity against multiple and otherwise unrelated VHFs, including Ebola virus (a Filovirus), Rift Valley Fever (a Bunyavirus) and Dengue virus (a Flavivirus). Exploratory testing also revealed potential inhibitory activity against additional viral pathogens including HIV (a retrovirus) and hepatitis C virus (a Flavivirus).

2. Materials and methods

2.1. Screening for viral inhibition of GFP-ZEBOV

A focused small molecule library was developed at USAMRIID, which consisted of compounds sharing chemical scaffolds with heterocyclic aromatic structures and possessing two positive-ionizable amidine or imidazolino moieties. These compounds were initially screened for antiviral activity in a cell-based high throughput

* Corresponding author.
E-mail address: mkinch@functional-genetics.com (M.S. Kinch).
[1] These authors contributed equally to the manuscript.
[2] Current address: Integrated Biotherapeutics, 20358 Seneca Meadows Parkway, Germantown, MD 20876, United States.

0166-3542/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2009.06.001

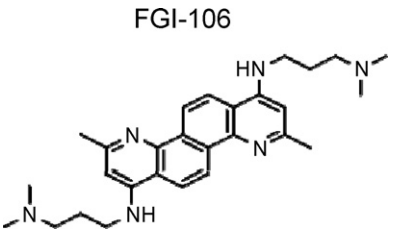

Fig. 1. Structure of FGI-106. Shown is the chemical structure of Quino[8,7-h]quinoline-1,7diamine,N,N′-bis[3-(dimethylamino)propyl]-3,9-dimethyl-, tetrahydrochloride (FGI-106), which was identified in a screen of small molecule compounds for activity against Ebola virus in cell-based assays.

screening (HTS) assay using a recombinant Zaire EBOV (GFP-ZEBOV) expressing green fluorescent protein (GFP) as a reporter for virus replication (Towner et al., 2005). The primary hits comprised molecules that decreased Ebola replication by at least 60%. One particular compound, Quino[8,7-h]quinoline-1,7diamine,N,N′-bis[3-(dimethylamino)propyl]-3,9-dimethyl-tetrahydrochloride (designated hereafter as FGI-106; Fig. 1), is the focus of our present report.

A recombinant Zaire EBOV expressing green fluorescent protein (GFP-ZEBOV) as a reporter for virus replication was used to screen compounds for inhibitors of EBOV replication. Briefly, confluent Vero E6 cells (96-well plate format) were pretreated with compound (0–20 μM) diluted in cell-culture medium for 18 h. This pretreatment was part of a standard protocol used for our initial screening of compounds but subsequent studies demonstrated that pretreatment was not required to obtain antiviral activity. Each plate included negative (DMSO; Cat # D2650; Sigma–Aldrich, St. Louis, MO) control treatments. Following pretreatment with compound, cells were infected with GFP-ZEBOV (multiplicity of infection (MOI) = 1). Compound was reapplied to cells after infection by adding additional media containing compound concentrations equivalent to pretreatment concentrations. Cultures were incubated for 48 h before fixation (10% neutral-buffered formalin) and removal from the BSL4 facility. After nuclear staining (Hoechst dye), viral infection was determined using a high content imaging system, Discovery 1 (Molecular Devices, Sunnyvale, CA), which compiles GFP fluorescence data from approximately 25,000 cells per well. This procedure identifies the total number of adherent cells, and fraction of infected cells, thereby providing an immediate assessment of efficacy and toxicity. Cell viability assays were conducted as detailed previously (Kinch et al., 2009). All assays were repeated at least three times and representative findings are shown.

2.2. Virus yield reduction assay

All products used for cell culture were obtained from Invitrogen. Confluent Vero E6 cells (American Type Culture Collection, Manassas, VA) in 24-well plates were treated with FGI-106 diluted in cell-culture medium or with medium only (negative control). Following overnight incubation, medium was removed and cells were infected with Zaire EBOV (ZEBOV; MOI = 1). After 1 h, excess virus was removed and media containing FGI-106 at pretreatment concentrations was replenished. Culture supernatants were collected after 48 h and viral titers were quantified by standard plaque assay using Vero E6 cells. The plaque assays for Rift Valley Fever virus (MP-12; MOI = 1) were conducted after 24 h of infection in the presence of the indicated amounts of FGI-106. To evaluate Dengue Fever virus, DC-Sign Raji cells were infected with Dengue (isolates DEN1, DEN2, DEN3 or DEN4; MOI = 0.1) in the presence or absence of compound for 72 h as previously reported (Sun et al., 2009). Flow cytometric assessment of cell staining with 2H2 monoclonal antibody (Sun et al., 2009), which recognizes a Dengue complex-specific antigen, provided an assay of infection with Dengue Fever virus (Mady et al., 1991). All assays were repeated at least three times and representative findings are shown.

2.3. Evaluation of HCV

To evaluate release of infectious HCV particles, human hepatoma cells (Huh-7; purchased from ATCC, Manassas, VA) were infected with a recombinant hepatitis C virus (HCV) that expresses a *renilla* luciferase at IP Pharmaceuticals (Lexington, KY). At 2 h post-infection, HCV-infected cells were incubated with compounds at concentrations ranging from 30 to 0.04 μM in a 3-fold serial dilution (30, 10, 3.33, 1.11, 0.37, 0.12, and 0.04 μM). At 3 days post-infection, cells were lysed in a buffer included in a Promega luciferase assay kit. The level of luciferase expression was determined by luciferase assay (Promega Corp., Madison, WI). To evaluate reduction of HCV protein, Huh-7 cells were infected with HCV. At 2 h post-infection, HCV was removed and cells were washed once with PBS. HCV-infected cells were then incubated with inhibitors at the indicated concentrations at 37 °C for 3 days. Cell lysate was prepared by extracting cells in 50 μl RIPA buffer. The level of HCV NS5A protein was determined by Western blot analysis using an NS5A-specific monoclonal antibody.

2.4. In vitro testing of HIV

MT-4 cells (purchased from ATCC, Manassas, VA) were infected with HIV-1 NL4–3 (obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH) at an MOI of 0.001 by low speed centrifugation ($1200 \times g$) for 1 h (Adachi et al., 1986). Cells were seeded in a 96-well plate (1.5×10^5 in 100 μl culture medium per well) (Wei et al., 2002). Serial dilution of FGI-106 was immediately added in triplicate in 50 μl culture medium per well. Half of the supernatants were refreshed every day starting from day 3 pi in the presence of same concentration of FGI-106. The collected supernatants were then transferred to the TZM-bl indicator cell line (obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH) for examination of viral production in FGI-106 treated samples. Relative Luminescence Unit (RLU) was obtained on TZM-bl cells after they were treated with Bright-Glo Luciferase Assay System (Promega) 3 days later. The percentages of inhibition of viral production by FGI-106 were calculated as: (RLU from mock-treated samples − RLU from FGI-106 treated samples)/RLU from mock-treated samples × 100. Normal MT-4 cells were treated with serial dilution of FGI-106 as same as above and its cytotoxicity was measured by CytoTox96 Non-Radioactive Cytotoxicity Assay (Promega) according to the Manufacturer's instruction.

2.5. Mouse efficacy studies

Male or female C57BL/6 or BALB/c mice were obtained from the National Cancer Institute, Frederick Cancer Research and Development Center (Frederick, MD) and were used at 6–10 weeks of age at the initiation of experiments. Mice were housed in microisolator cages and were provided water and chow *ad libitum*. FGI-106 was delivered to mice ($n = 10$ per treatment group) by intraperitoneal (IP) injection in a vehicle of 0.9 percent saline. To challenge mice, 1000 plaque forming units (pfu; $3000\times$ LD_{50}) of mouse-adapted EBOV (Bray et al., 1998) was delivered by IP injection under biosafety level 4 containment at the United States Army Medical Research Institute for Infectious Diseases. Animal treatment adhered to principles described in Guide for the Care and use of Laboratory Animals, National Research Council, 1996. The mouse studies were performed at least six times and representative findings are shown. For studies using a prophylaxis setting, the mice were treated with drug, administered intraperitoneally, 2 h

prior to challenge with Ebola virus and then again 2 and 5 days post-challenge. For studies using a therapeutic setting, the animals were treated 1, 2 or 3 days post-infection as indicated in the Figure Legends. To evaluate the statistical significance, the samples were subjected to Kaplan Meier survival analysis and log-rank tests with stepdown Bonferroni adjustment to compare survival curves among groups.

To evaluate EBOV organ viral burdens in mice, animals were euthanized by CO_2 asphyxation, and kidney, spleen, and liver samples were harvested, weighed, and homogenized in cell-culture medium. Homogenized tissues were centrifuged and the supernatant was stored at −80 °C. Supernatants were subjected to standard plaque assay using Vero E6 cells.

To evaluate pharmacokinetic parameters, male C57BL/6 mice were administered a dosing solution of 3 mg/kg FGI-106 intravenously. The subjects were sacrificed at the times indicated (0, 5, 15, 30, 60, 180 and 300 min; $n = 3$ per group) and plasma samples were analyzed by mass spectrometry at Absorption Systems, LP (Exton, PA). The experimental readouts included maximum plasma concentration, half-life, area under the curve (calculated to the last available time point, $t = 300$ min) and an estimated area under the curve (extrapolated to infinity). For studies of organ accumulation, tissues samples were harvested from liver, kidney and spleen and subjected to the same mass spectrometric investigation. Please note organ accumulation data are reported as mg/kg, whereas plasma levels are reported as mg/mL.

3. Results

3.1. Identification of FGI-106, a small molecule active against Ebola virus

The FGI-106 compound was selected based on its ability to consistently inhibit Ebola virus replication in a dose-dependent manner. The antiviral activity of FGI-106 was originally described in Section 2 and its antiviral activity was confirmed using Ebola virus plaque enumeration as a direct measure of viral replication (Fig. 2A). These studies revealed that treatment with 2 μM FGI-106 mediated a 4 log reduction in infectious viral titers relative to matched controls, with an EC_{90} for inhibition of viral killing of host cells estimated to be 0.6 μM.

We then considered the possibility that the antiviral activity observed herein might simply reflect toxicity to the host cells. Additional investigation sought to determine the level of drug, which would cause general cytotoxicity (independent of viral infection). In vitro toxicity testing revealed that toxicity to Vero E6 cells was observed at higher concentrations than was observed for antiviral activity (Fig. 2B). For example, the concentration of drug required for cellular cytotoxicity (CC_{50}) for Vero E6 was estimated to be 10 μM, whereas antiviral activity was observed at much lower doses. Likewise, FGI-106 was not toxic to other host cells utilized herein, including Marc-145 ($CC_{50} > 50$ μM), PK-15 ($CC_{50} > 25$ μM) DC-Sign ($CC_{50} > 20$ μM) and MT-4 ($CC_{50} = 6$ μM).

3.2. Broad-spectrum antiviral activity of FGI-106

Given the need for new therapeutics for VHFs, cell-based assays were conducted to screen FGI-106 against a panel of unrelated VHF viruses, including Rift Valley Fever virus (RVFV) and Dengue Fever virus (Fig. 3A and B). FGI-106 also inhibited non-hemorrhagic fever viruses, including hepatitis C virus and human immunodeficiency virus (HIV) (Fig. 3C and D and data not shown). Similar to the findings with Ebola virus, the EC_{90} values for these viruses were much lower than the levels of drug that caused toxicity to the host cells (defined as the CC_{50}), thus precluding that these

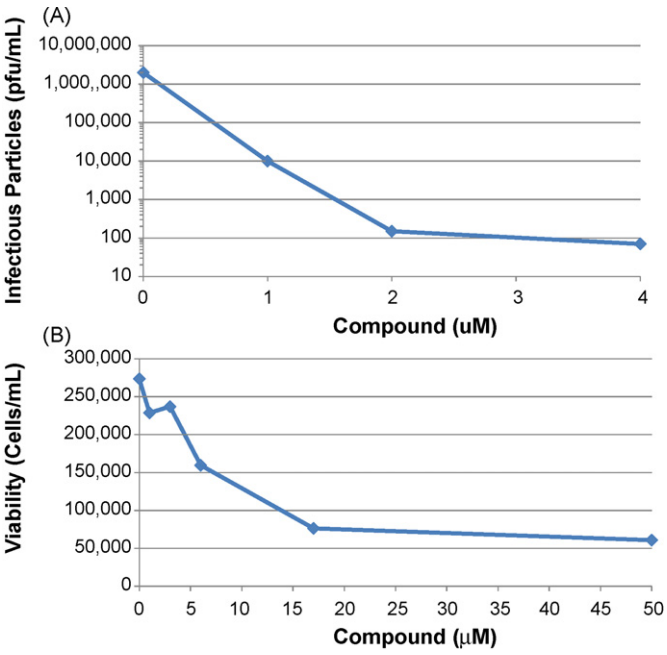

Fig. 2. FGI-106 blocks Ebola virus infection. (A) Vero E6 cells were infected with Zaire-Ebola virus (ZEBOV) for 72 h in the presence of 0–4 μM FGI-106. Antiviral efficacy was evaluated using plaque assays of infectious Ebola particles to assess the number of plaque forming units per unit volume (pfu/mL). (B) The number of viable Vero E6 cells was assessed following a 72 h incubation in the presence of the indicated concentrations of FGI-106. Viability was assessed using an automated cell counter.

agents blocked viral production simply through toxic effects to the host (summarized in Table 1). The cell systems under investigation included those isolated from solid tissues (Vero E6, Huh-7) or lymphoid cells (MT-4, DC-Sign) as well as cells derived from human or non-human sources. Altogether, these findings suggest FGI-106 possesses broad-spectrum activity against a wide array of different VHF pathogens and host cell types.

3.3. Efficacy of FGI-106 in mouse models of Ebola virus infection

While the broad-spectrum antiviral activity in cell-based assays was intriguing, animal-based efficacy represents a greater challenge. Prior to initiating investigation of FGI-106 in mice, it was necessary to determine the bioavailability of the molecule, particularly within relevant target organs. Since our goal was to focus on Ebola virus, a panel of pharmacokinetic studies were conducted to evaluate serum levels of FGI-106 as well as accumulation within organs that are normally targeted by Ebola virus: kidney, liver and spleen. FGI-106 was administered intravenously at 3 mg/kg to C57BL/6 mice and serum samples collected over time (at 0, 5, 15, 30, 60, 180 and 300 min) for analysis of FGI-106 levels using tandem mass spectrometry. These studies revealed maximal concentration 5 min after injection, with an estimated serum half-life of approximately 1.8 h (see Fig. 4A). This rapid depletion from serum led us to ask if FGI-106 might efficiently distribute into the spleen, liver, and kidney. For this, groups of three C57BL/6 mice received a 3 mg/kg intravenous dose of FGI-106 for 6 h. This particular time point was selected based on prior experience that 6 h is sufficient time to allow a drug to transit from the blood and accumulate within organs. The animals were sacrificed and the lung, liver, kidney and spleen harvested for mass spectrometry-based assessment of FGI-106 concentrations. These studies revealed that the compound had entered organs at levels ranging from 19.5 (spleen) to 43.1 μg/g (kidney) (Fig. 4B). Such findings suggested potential promise for efficacy in animals in light of

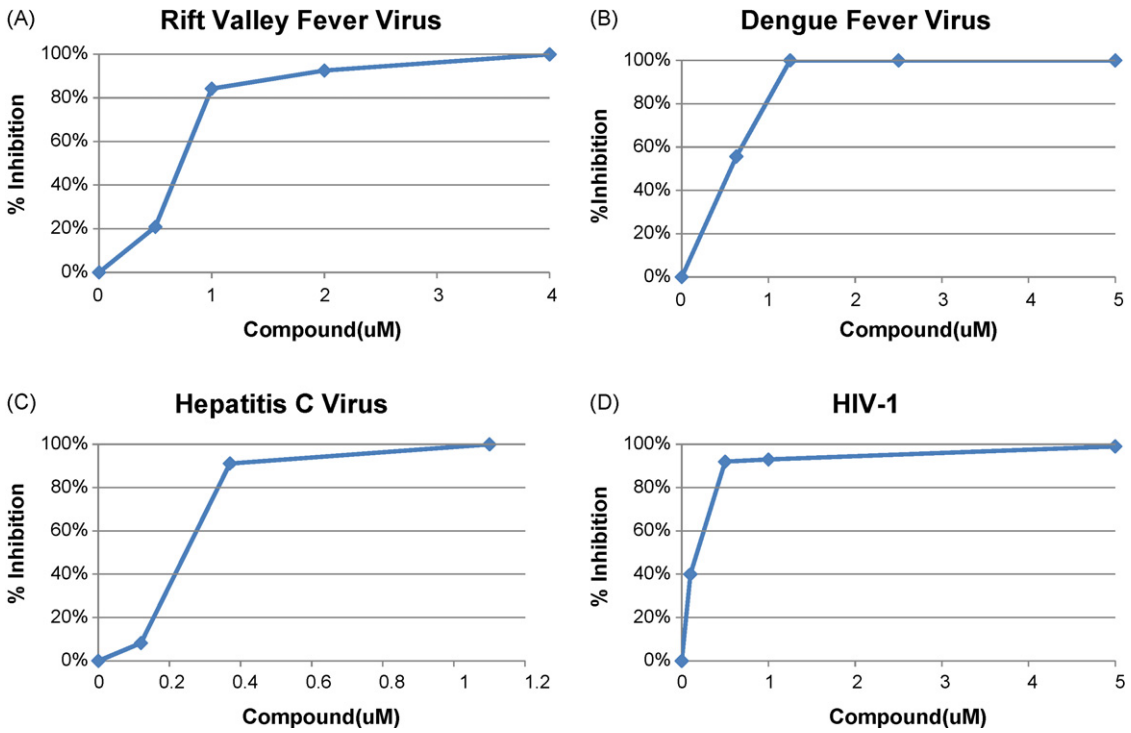

Fig. 3. Broad-spectrum antiviral activity by FGI-106 against RNA viruses among different families. The antiviral activity of FGI-106 was assessed using cell-based assays of infection by (A) Rift Valley Fever virus (strain MP-12), (B) Dengue Fever virus (DEN2), (C) Hepatitis C virus or (D) HIV-1. Note the assays employed to evaluate FGI-106 necessarily varied when evaluating different virus types (see Table 1 for details).

cell-based assays, which had estimated an EC_{90} of 0.004 μg/g for Ebola virus.

To evaluate the antiviral activity of FGI-106 in mouse models, Ebola virus was selected based on the unmet needs for an effective antiviral for Ebola virus and the availability of animal models. In our first set of studies, C57BL/6 mice were infected with a mouse-adapted strain of Zaire EBOV (Bray et al., 1998) (known hereafter as MA-ZEBOV). Animals were treated with different doses of FGI-106 and challenged after an hour with Ebola virus (1000 pfu/animal, 3000× LD_{50}) followed by two additional injections of FGI-106 at 24 and 72 h post-infection. These time points were selected to maintain drug exposure throughout the study. This prophylaxis model increased the likelihood that drug was available to the host prior to infection. Due to safety constraints when working in a BSL4 setting, we were limited to treating animals *via* intraperitoneal injection. Treatment with FGI-106 decreased mortality from MA-EBOV in a dose-dependent manner (Fig. 5). For example, treatment with 2 ($p < 0.0002$) or 5 mg/kg ($p < 0.0002$) was sufficient to protect animals from Ebola virus. Lower doses of FGI-106 treatment decreased

Table 1
Overview of broad-spectrum antiviral activity of FGI-106.

Virus	EC_{50}	EC_{90}	Host cell	CC_{50}	Selectivity index
Ebola	100 nM	0.5 μM	Vero E6	10 μM	100
Rift Valley Fever	800 nM	2 μM	Vero E6	10 μM	12
Dengue, Type 1	400 nM	1.1 μM	DC-Sign	20 μM	50
Dengue, Type 2	400 nM	1.0 μM	DC-Sign	20 μM	50
Dengue, Type 3	900 nM	2.3 μM	DC-Sign	20 μM	22
Dengue, Type 4	400 nM	1.1 μM	DC-Sign	20 μM	50
HCV	200 nM	0.4 μM	Huh-7	5 μM	25
HIV-1	150 nM	0.3 μM	MT-4	6 μM	40

Shown is an overview of cell-based studies to evaluate the antiviral efficacy (EC_{90}) and overall toxicity (CC_{50}) of FGI-106. Note that the antiviral activity of FGI-106 was observed using multiple and unrelated viruses and with different cell types and species. The selectivity index represents the CC_{50}/EC_{50}.

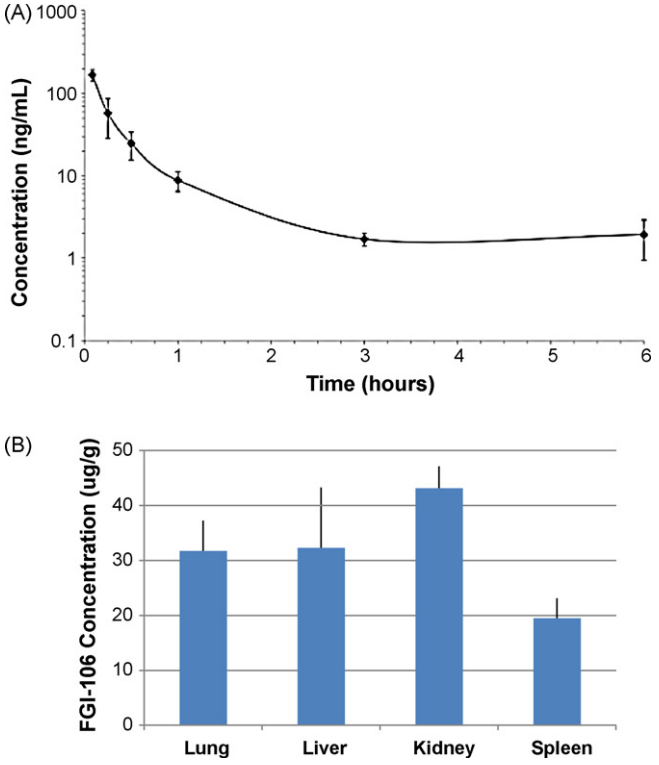

Fig. 4. Pharmacokinetic analysis of FGI-106. C57BL/6 mice were treated with 3 mg/kg of FGI-106, administered intravenously. (A) Serum samples were harvested 0–6 h post-treatment. The samples were then analyzed using tandem mass spectrometry FGI-106 serum concentrations assessed over time. (B) Vital organs were harvested from sacrificed subjects 24 h post-infection and FGI-106 levels were measured in homogenized samples using mass spectrometry.

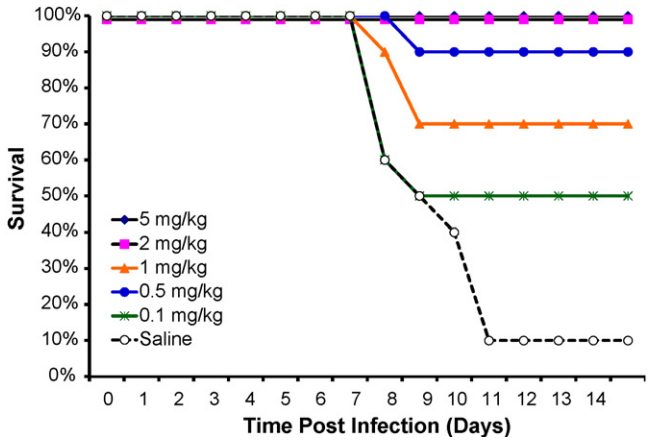

Fig. 5. Treatment with FGI-106 prevents Ebola virus lethality when modeled in a prophylactic setting. C57BL/6 mice were treated with FGI-106, administered via intraperitoneal injection at the concentrations shown, and were then challenged by 1000 pfu (3000× LD_{50}) of mouse-adapted Ebola virus. Survival was then assessed daily for the next 2 weeks, with animals receiving additional treatments on days 2 and 5. A vehicle control, prepared and administered identically to that for FGI-106, provided a matched negative control for treatment (open circles, dotted lines). Note that the results from treatment with either 2 or 5 mg/kg were overlapping and conferred 100% protection in this study). Statistical analyses revealed significant inhibition of Ebola-mediated death in animals treated with 5 or 2 mg/g ($p < 0.0002$ for both), 1 mg/kg ($p = 0.029$) and 0.5 mg/kg ($p = 0.0018$) relative to saline-treated controls.

survival. For example, subjects treated with 1 mg/kg FGI-106 had a mean survival time of 12.40 ± 0.95 days ($p = 0.029$) and 0.5 mg/kg treatment resulted in a mean survival time of 13.4 ± 1.03 days ($p = 0.0018$) as compared with vehicle-treated controls (Mean survival of 9.8 ± 0.63 days). Treatment with 0.1 mg/kg FGI-106 (mean survival of 11.1 days; $p = 0.1726$) did not significantly improve survival following Ebola virus challenge. Consistent with our earlier findings, prophylactic treatment with FGI-106 treatment also protected BALB/c mice whereas a matched vehicle control did not (Fig. 6, blue diamonds versus open diamonds, respectively).

We progressively increased the stringency of our efficacy studies by delaying treatment until after viral infection. Using this therapeutic model, FGI-106 treatment was initiated 1 day after a

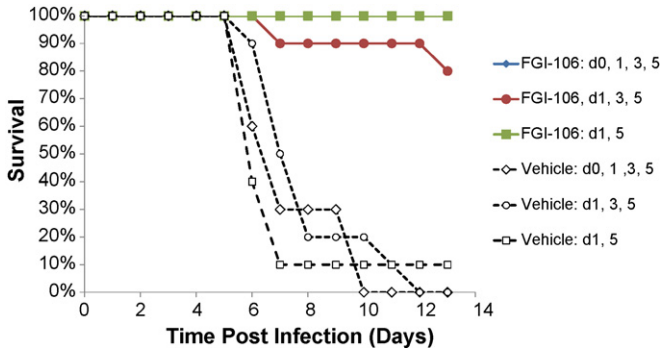

Fig. 6. FGI-106 prevents Ebola virus lethality when modeled in a therapeutic setting. BALB/c mice were treated with FGI-106, administered intraperitoneally at 5 mg/kg on the days shown (closed, colored symbols), and were then challenged by intraperitoneal injection with 1000 pfu (3000× LD_{50}) of mouse-adapted Ebola virus. Survival was then assessed daily for the next 2 weeks. Vehicle control, prepared and administered identically to that for FGI-106, provided matched negative controls for treatment (open symbols). Note that the first two treatment groups listed (d0,1,3,5 (blue diamonds) and d1,3,5 (green squares)) both conferred 100% protection from an otherwise lethal challenge with Ebola virus. Statistical analyses revealed significant inhibition of Ebola-mediated death in all groups treated with FGI-106 relative to vehicle-treated controls ($p < 0.0001$ for each comparison). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

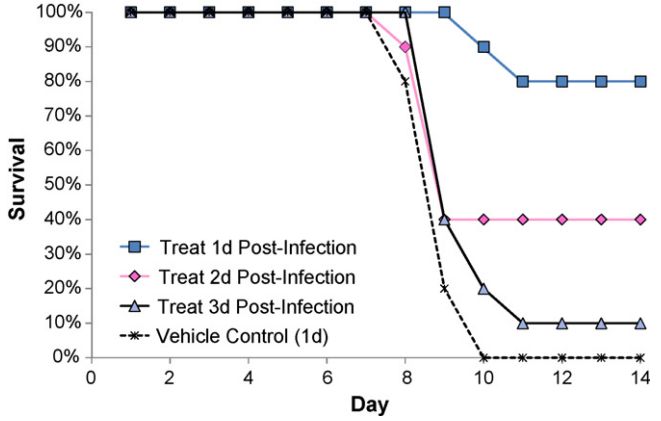

Fig. 7. Delay of treatment initiation relates to survival. The time of treatment initiation with FGI-106 was delayed for 0–3 days following infection with intraperitoneal challenge with mouse-adapted Ebola virus (1000 pfu, 3000× LD_{50}). Survival was assessed daily and injection with a vehicle (injected 1 day post-infection) provided a negative control. Statistical analyses revealed significant inhibition of Ebola-mediated death in animals treated 1 day post-infection ($p < 0.0001$) but delaying treatment further resulted only in trends towards increased survival ($p < 0.1276$).

lethal challenge with 1000 pfu (3000× LD_{50}) of MA-EBOV. FGI-106 treatment conferred protection from Ebola virus (mean survival time of 13.3 ± 0.63 days), even under these therapeutic conditions (Fig. 6, red circles and green squares). In a further escalation of stringency, mice were treated with a single dose of FGI-106, administered 1, 2, or 3 days after a lethal challenge (1000 pfu; 3000× LD_{50}). A single dose of FGI-106 administered 1 day post-infection sufficed to confer protection. Fig. 7; $p < 0.0001$ for each FGI-106 treatment group relative to its matched vehicle control group). When delayed to 2 days post-infection, treatment trended towards increased survival (mean survival of 10.9 ± 0.88 days as compared with vehicle-treated controls, 9.0 days), but this effect was not statistically significant ($p = 0.1276$; Fig. 7). Likewise, when initiation of treatment was delayed to 3 days post-infection, FGI-106 treatment did not decrease lethality (mean survival time of 9.90 ± 0.21 days; $p = 0.1276$).

We further escalated the stringency of the experimental conditions by asking if a single dose of FGI-106, administered 24 h post-infection, would be sufficient to protect animals from a lethal challenge with Ebola virus. Indeed, a single dose of FGI-106 (5 mg/kg) conferred protection and in a dose-dependent manner (Fig. 8A). To examine this outcome in greater detail, a parallel series of studies were conducted in which subjects were sacrificed 3 days post-infection. This particular time point was selected since it would allow us to evaluate viral propagation prior to the onset of mortality, which generally starts 4–6 days post-infection and known sites of Ebola virus proliferation (kidney, spleen, liver) were harvested (Fig. 8B). Consistent with the survival of animals treated with FGI-106 (a single dose of 5 mg/kg administered 1 day post-infection), plaque analyses demonstrated a dramatic reduction in MA-EBOV in the liver, spleen and kidney of FGI-106 treated-animals relative to matched controls.

4. Discussion

There are currently no antivirals to treat Ebola virus infection in humans, which can cause 40–90% mortality (Zampieri et al., 2007). The major finding of our present study is the identification of FGI-106, a novel compound that confers protection from Ebola virus when evaluated within either a prophylactic or therapeutic setting. We also demonstrate that FGI-106 demonstrates activity against multiple and genetically distinct viruses.

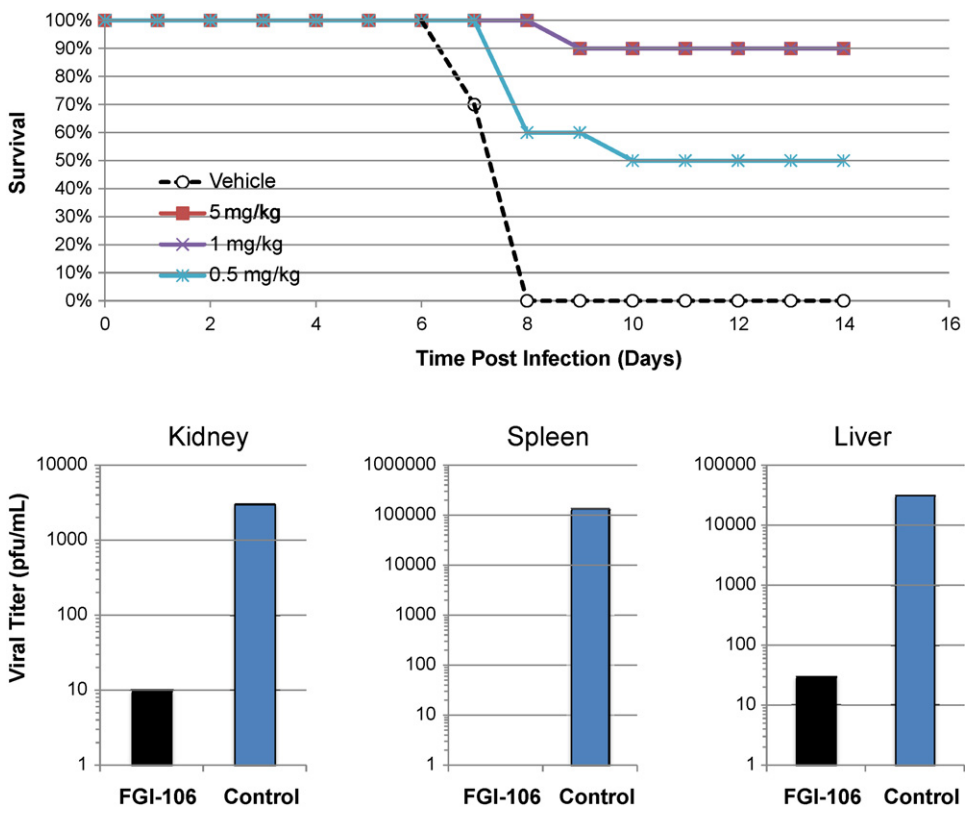

Fig. 8. A single dose of FGI-106 protects animals from Ebola in a therapeutic setting. The same therapeutic model for Ebola virus as utilized in Fig. 4 was investigated by delaying the treatment with a single dose of FGI-106 until 24 h post-infection. (A) C57BL/6 mice were treated with FGI-106, administered intraperitoneally, 24 h post-infection and survival was assessed over time. Vehicle-treated animals (open circles, dotted lines) provided a matched negative control. (B) In a parallel study, a subset of BALB/c mice that had been treated with a single dose of 5 mg/kg FGI-106 1 day post-infection, were sacrificed at 3 days post-infection (prior to the onset of morbidity and mortality) and the number of infectious Ebola particles was measured within critical target organs (kidney, spleen, liver) using standard plaque assays.

One feature of the present finding is the demonstration of broad-spectrum antiviral activity. In cell-based assays, treatment with FGI-106 inhibited viral replication by divergent virus families, including positive and negative-strand RNA viruses. Based on this range of activity we suspect the compound may target a common host factor required for replication of diverse virus families. This outcome would be consistent with an emerging concept of host-oriented therapeutics, which advocates a strategy of safely targeting critical host mechanisms that are essential of the virus, but not for normal host cell function or survival.

Based on the broad-spectrum nature of the antiviral activity, we postulate the mechanistic basis of FGI-106 antiviral activity involves a conserved host pathway. For example, comparable efficacy was observed using human, mouse or primate cells, suggesting the antiviral effects of FGI-106 are not unique to a particular cell type or species. The broad-spectrum nature of FGI-106 antiviral activity may suggest FGI-106 targets a fundamental and highly conserved host mechanism. The concept of host-based targeting is not unique to the finding herein. For example, ribavirin exerts its effects in part by inhibiting the host cell enzyme, IMP dehydrogenase (Franchetti and Grifantini, 1999; Goldstein and Colby, 1999; Robins et al., 1985). Likewise, inhibitors of host-encoded S-adenosylhomocysteine inhibit methylation of viral mRNA cap structures (Hunt, 1989; Rose et al., 1977). Although drugs like Ribavirin have been utilized to suppress the replication of a wide range of viruses, it is associated with toxicity (Johnson, 1990; Kumar et al., 2002). Future investigation will be important to identify the molecular basis by which FGI-106 conveys its robust antiviral activity. Once the targets and pathways have been identified, it will be important to determine the potential for drug-based toxicity, which could impact its ultimate application in the clinic.

Conventional antiviral approaches target viral molecules to minimize host toxicity (De Clercq, 2008). However, the combination of a high replication rate and an error prone polymerase (lacking proofreading activity) favors selection of drug-resistant variants. We hypothesize that host-directed therapeutics will not be as susceptible to such resistance mechanisms. Consistent with this idea, no virus has been reported to develop resistance to ribavirin, which acts through a host-based mechanism. Moreover, host-based targeting strategies in general and FGI-106 in particular, could provide an unprecedented opportunity to deploy a therapeutic option with application to many different virus types. A broad-spectrum antiviral could be particularly useful under conditions where there is insufficient time or ability to identify the causative pathogen. Further investigation will be important to address the potential for FGI-106 and other host-based antivirals and such findings could have for the development of novel therapeutic options that are broad-spectrum in their application and durable in antiviral activity.

Acknowledgement

This work was supported by the Transformation Medical Technologies Initiative (TMTI) program of the Defense Threat Reduction Agency (DTRA).

References

Adachi, A., Gendelman, H.E., Koenig, S., Folks, T., Willey, R., Rabson, A., Martin, M.A., 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. 59, 284–291.

Barreto, M.L., Teixeira, M.G., 2008. Dengue fever: a call for local, national, and international action. Lancet 372, 205.
Bray, M., 2008. Highly pathogenic RNA viral infections: challenges for antiviral research. Antiviral Res. 78, 1–8.
Bray, M., Davis, K., Geisbert, T., Schmaljohn, C., Huggins, J., 1998. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. J. Infect. Dis. 178, 651–661.
Burnett, J.C., Henchal, E.A., Schmaljohn, A.L., Bavari, S., 2005. The evolving field of biodefence: therapeutic developments and diagnostics. Nat. Rev. Drug Discov. 4, 281–297.
De Clercq, E., 2008. Antivirals: current state of the art. Future Virol. 3, 393–405.
Franchetti, P., Grifantini, M., 1999. Nucleoside and non-nucleoside IMP dehydrogenase inhibitors as antitumor and antiviral agents. Curr. Med. Chem. 6, 599–614.
Goldstein, B.M., Colby, T.D., 1999. IMP dehydrogenase: structural aspects of inhibitor binding. Curr. Med. Chem. 6, 519–536.
Hensley, L.E., Jones, S.M., Feldmann, H., Jahrling, P.B., Geisbert, T.W., 2005. Ebola and Marburg viruses: pathogenesis and development of countermeasures. Curr. Mol. Med. 5, 761–772.
Hunt, D.M., 1989. Effect of analogs of S-adenosylmethionine on invitro polyadenylation by vesicular stomatitis virus. J. Gen. Virol. 70, 535–542.
Johnson, E.M., 1990. Developmental toxicity and safety evaluations of ribavirin. Pediatr. Infect. Dis. J. 9, S85–S87.
Khan, S.H., Goba, A., Chu, M., Roth, C., Healing, T., Marx, A., Fair, J., Guttieri, M.C., Ferro, P., Imes, T., Monagin, C., Garry, R.F., Bausch, D.G., 2008. New opportunities for field research on the pathogenesis and treatment of Lassa fever. Antiviral Res. 78, 103–115.
Kinch, M.S., Yunus, A.S., Lear, C., Mao, H., CHen, H., Fesseha, Z., Luo, G., Nelson, E.A., Li, L., Huang, Z., Murray, M., Ellis, W.Y., Hensley, L.E., Christopher-Hennings, J., Olinger, G.G., Goldblatt, M., 2009. FGI-104: a broad-spectrum small molecule inhibitor of viral infection. Am. J. Transl. Res. 1, 87–98.
Kumar, K.S., Russo, M.W., Borczuk, A.C., Brown, M., Esposito, S.P., Lobritto, S.J., Jacobson, I.M., Brown, R.S., 2002. Significant pulmonary toxicity associated with interferon and ribavirin therapy for hepatitis C. Am. J. Gastroenterol. 97, 2432–2440.
Mady, B.J., Erbe, D.V., Kurane, I., Fanger, M.W., Ennis, F.A., 1991. Antibody-dependent enhancement of dengue virus infection mediated by bispecific antibodies against cell surface molecules other than Fc gamma receptors. J. Immunol. 147, 3139–3144.
Marty, A.M., Jahrling, P.B., Geisbert, T.W., 2006. Viral hemorrhagic fevers. Clin. Lab. Med. 26, 345–386.
Robins, R.K., Revankar, G.R., McKernan, P.A., Murray, B.K., Kirsi, J.J., North, J.A., 1985. The importance of IMP dehydrogenase inhibition in the broad spectrum antiviral activity of Ribavirin and Selenazofurin. Adv. Enzyme Regul. 24, 29–43.
Rose, J.K., Lodish, H.F., Brock, M.L., 1977. Giant heterogeneous polyadenylic acid on vesicular stomatitis virus mRNA synthesized in vitro in presence of S-adenosylhomocysteine. J. Virol. 21, 683–693.
Sun, P.F., Fernandez, S., Marovich, M.A., Palmer, D.R., Celluzzi, C.M., Boonnak, K., Liang, Z.D., Subramanian, H., Porter, K.R., Sun, W., Burgess, T.H., 2009. Functional characterization of ex vivo blood myeloid and plasmacytoid dendritic cells after infection with dengue virus. Virology 383, 207–215.
Towner, J.S., Paragas, J., Dover, J.E., Gupta, M., Goldsmith, C.S., Huggins, J.W., Nichol, S.T., 2005. Generation of eGFP expressing recombinant Zaire ebolavirus for analysis of early pathogenesis events and high-throughput antiviral drug screening. Virology 332, 20–27.
Wei, X.P., Decker, J.M., Liu, H.M., Zhang, Z., Arani, R.B., Kilby, J.M., Saag, M.S., Wu, X.Y., Shaw, G.M., Kappes, J.C., 2002. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob. Agents Chemother. 46, 1896–1905.
Zampieri, C.A., Sullivan, N.J., Nabel, G.J., 2007. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nat. Immunol. 8, 1159–1164.