Merimepodib, an IMPDH inhibitor, suppresses replication of Zika virus and other emerging viral pathogens

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1. Introduction

Zika virus (ZIKV), a member of the Flaviviridae family, has recently been linked to abnormal pregnancies, fetal death, microcephaly, and Guillain-Barré syndrome in humans. Merimepodib (MMPD, VX-497), a potent inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH), has shown antiviral activity against HCV and a variety of DNA and RNA viruses in vitro. In this report, we expand the antiviral spectrum of MMPD, and demonstrate that MMPD inhibits ZIKV RNA replication with an EC\textsubscript{50} of 0.6 \mu M. Furthermore, MMPD reduces the virus production of ZIKV as well as several other important emerging viral pathogens such as Ebola, Lassa, Chikungunya, and Junin viruses. The inhibition can be reversed by addition of exogenous guanosine to culture media, consistent with the mechanism of action of MMPD as an IMPDH inhibitor. We also provide evidence that MMPD can be used in combination with other antivirals such as ribavirin and T-705 (favipiravir) to enhance suppression of virus production.

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\textbf{ABSTRACT}

Zika virus (ZIKV), a member of the Flaviviridae family, has recently been linked to abnormal pregnancies, fetal death, microcephaly, and Guillain-Barré syndrome in humans. Merimepodib (MMPD, VX-497), a potent inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH), has shown antiviral activity against HCV and a variety of DNA and RNA viruses in vitro. In this report, we expand the antiviral spectrum of MMPD, and demonstrate that MMPD inhibits ZIKV RNA replication with an EC\textsubscript{50} of 0.6 \mu M. Furthermore, MMPD reduces the virus production of ZIKV as well as several other important emerging viral pathogens such as Ebola, Lassa, Chikungunya, and Junin viruses. The inhibition can be reversed by addition of exogenous guanosine to culture media, consistent with the mechanism of action of MMPD as an IMPDH inhibitor. We also provide evidence that MMPD can be used in combination with other antivirals such as ribavirin and T-705 (favipiravir) to enhance suppression of virus production.

1. Introduction

Zika virus (ZIKV) is a member of the Flaviviridae family, which also includes several pathogens of clinical significance such as dengue and hepatitis C (HCV) viruses. Since 2007, ZIKV has spread from Oceania and was first detected in Brazil in early 2015. Within a year, it had propagated across Latin America and has now reached the United States. Historically, ZIKV infection causes a mostly self-limiting disease in humans, ranging from no signs to mild symptoms which are similar in the early stages to those associated with other common arboviruses.

More recently, ZIKV infections have been linked to neurological disorders such as Guillain-Barré syndrome (GBS) in adults, as well as abnormal pregnancies, fetal death, and brain defects in newborns due to the transmission of ZIKV from a pregnant woman to her fetus (Chan et al., 2016; Lazear and Diamond, 2016; Lessler et al., 2016). Upon systemic infection, ZIKV replication can be detected in many organs, including several immune-privileged sites such as the brain (Wang et al., 2016), placenta (Quicke et al., 2016; Miner et al., 2016a) and eyes (Miner et al., 2016b). In humans, persistent shedding of infectious virus has been found in vaginal secretions (Lustig et al., 2016) and semen (Moreira et al., 2017) after the viremic and symptomatic stage of virus infection. The unexpected spread and clinical symptoms prompted the World Health Organization to declare ZIKV a Public Health Emergency of International Concern from February to November 2016. To date, no ZIKV specific vaccines and therapeutics have been approved for clinical use, although several vaccines candidates are under clinical development (Cohen, 2016; Pierson and Graham, 2016).

In the past decade, other important viral pathogens from diverse families have emerged (or re-emerged) to cause serious outbreaks (Bray, 2008; Weaver and Reisen, 2010; Dash et al., 2013). Among them are Ebola virus (filovirus), Lassa and Junin virus (arenavirus), and Chikungunya virus (alphavirus). As some of these viruses co-circulate in tropical and subtropical areas (e.g., Ebola and Lassa viruses in West Africa, Zika and Junin viruses in South America, and several arboviral viruses in Southeast Asia) and share certain early clinical symptoms (e.g., fever, headache, and fatigue), it can be difficult to provide rapid and accurate diagnosis (Roth et al., 2014; Tilak et al., 2016). In this context, a broad-spectrum antiviral would be highly desirable by alleviating the need for pretreatment diagnosis, especially in resource-limited countries where the outbreaks often originate. Furthermore, given the unpredictable nature of virus emergence and outbreak, the development of broad-spectrum antivirals could serve as a strategy for
global epidemic preparedness (Bekerman and Einav, 2015).

One mechanism to control rapidly replicating viruses is to manipulate the levels of cellular nucleoside triphosphate (NTP) pools. Inosine-5’-monophosphate dehydrogenase (IMPDH) is an enzyme involved in the de novo synthesis of guanine nucleotides (Hedstrom, 2009). Inhibitors of IMPDH such as ribavirin (RBV) and mycophenolic acid have shown activities against Chikungunya (Khan et al., 2011), Junin (Westover et al., 2016), Lassa, and Ebola viruses (Ölschläger et al., 2011). Merimepodib (MMPD, VX-497) is a potent and selective inhibitor of IMPDH (Sintchak and Nimmegsren, 2000). It has been shown to inhibit HCV and a variety of DNA and RNA viruses in vitro. The inhibition by MMPD can be reversed by addition of exogenous guanosine to culture media, which restores cellular GTP levels (Markland et al., 2000).

In this report, we aimed to expand the antiviral spectrum of MMPD, and demonstrated that MMPD inhibits ZIKV and several other globally emerging viral pathogens such as Ebola, Lassa, Chikungunya, and Junin viruses. We also provided evidence that MMPD can be used in combination with other antivirals to enhance suppression of virus production.

2. Materials and methods

2.1. Viruses, cell lines and test compounds

Virus strains: ZIKV strain FSS13025 was obtained from Dr. Scott Weaver at University of Texas Medical Branch (Galveston, TX); strain MR766 was obtained from BEI Resources (Manassas, VA). Ebola virus strain Zaire was obtained from Dr. Thomas Ksiazek at University of Texas Medical Branch. Lassa virus strain Josiah (Yin et al., 2013) and Junin virus strain Candid #1 (Emonet et al., 2011) were generated from molecular clones. Chikungunya virus strain SL15649 has been described in Morrison et al. (2011).

Cell lines: African green monkey kidney cells (Vero E6) were obtained from CDC (Atlanta, GA) or from Dr. Ilya Frolov at University of Texas Medical Branch. Cells were grown in DMEM (HyClone, Logan, UT) with 1% L-Glutamine (HyClone), 1x Penicillin/Streptomycin (HyClone), 1% MEM Vitamins (Lanza, Anaheim, CA), and supplemented with 10% FBS (Atlanta Biologicals, Flowery Branch, GA).

Test compounds: Merimepodib (MMPD) and favelipiravir (T-705) were purchased from Medchemexpress (Monmouth Junction, NJ). Ribavirin (RBV) was obtained from Cadila Healthcare (Ahmedabad, India). Compounds were dissolved in dimethyl sulfoxide (DMSO) and stored as 50 mM or 200 mM stock at −20 °C until use.

2.2. Cytotoxicity assays

Two assays for cytotoxicity were deployed. Quantitation of ATP cellular levels was performed using the CellTiter-Glo® Luminescent Cell Viability assay (Promega, Madison WI). Measurement of mitochondria function was performed using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (CellTiter-Glo®96 AQueous Reagent, Promega). Briefly, cells were seeded in 96-well plates for 24 h before being dosed with 1:3 serially-diluted compounds. Plates were harvested on time points as indicated and processed following manufacturer’s instructions.

2.3. Guanosine reversal

In guanosine reversal experiments, 100 μM guanosine (Sigma-Aldrich, St. Louis, MO) was added to growth media at the time of compound dosing.

2.4. Virus production assay

To measure the amount of virus produced, Vero cells were seeded in triplicates in 12-well plates at 2 × 10^5 cells/ml in order to reach 85–95% confluency in 24 h. Cells were infected with 0.1 MOI (multiplicity of infection) of viruses in 100 μl of infection media supplemented with 2% FBS. After 1 h incubation, the media were removed and fresh growth media containing compounds 1:3 serially diluted in DMSO were added to the wells. The final DMSO concentration in assay media was 0.5%. The media were replenished with fresh compound on Day 3. Aliquots (100 μl) of culture supernatants were collected at time points as indicated and virus titer was determined by plaque assays. The following virus strains were used: FSS13025 for ZIKV, Zaire for Ebola virus, Josiah for Lassa virus, and Candid #1 for Junin virus.

2.5. Virus plaque assay

To determine virus titer in collected supernatants, samples were serially diluted at a 1:10 dilution and used to infect 6-well plates of Vero cells. After incubation at 37 °C for 60–90 min, 0.5% SeaKem ME agarose (Lonza, Rockland, ME) overlay was added. On Day 5–7 post-inoculation, plates were fixed with 10% formaldehyde and stained with 0.25% crystal violet. The virus titer (log pfu/ml) and the reduction in the presence of test compounds relative to untreated samples were reported.

2.6. RT-PCR assay of Zika virus RNA

To measure the effect on ZIKV RNA level, Huh7 hepatoma cells (Southern Research, Frederick, Maryland) were infected with the strain MR766 at a MOI of 0.06. Serially diluted (1:3) compound was added to cells 12 h before infection. Viral RNA was purified from culture supernatants on Day 2 post infection, and the amount of viral RNA was measured by qRT-PCR. The qRT-PCR cycling conditions included an initial reverse transcription for 5 min at 53 °C, followed by 1 min at 95 °C and 45 cycles of 2-step cycling at 95 °C for 5s, then 60 °C for 50s. The genome copy numbers of ZIKV in culture supernatants were quantitated using a positive control RNA standard tested alongside on the same plate. Sequences of primer and probe from the NS1 region and additional experimental details have been described in Goebel et al. (2016). Cytotoxicity was assessed using the MIT cytotoxicity assay, EC_{50} (effective concentration inhibiting virus replication by 50%) and CC_{50} (effective concentration resulting in 50% cytotoxicity) values were calculated using GraphPad Prism 6 (GraphPad Software, La Jolla, CA). The selective index (SI) is defined as CC_{50}/EC_{50}.

2.7. Cytoprotection assay for Chikungunya virus

To determine the antiviral activity against Chikungunya virus in the cytoprotection assay, strain Sri Lanka (SL15649) and Vero cells were mixed in the presence of test compound and incubated for 3 days. The virus was pretitered such that control wells exhibit 85–95% loss of cell viability due to virus replication. The antiviral effect or cytoprotection was observed when compounds prevented virus replication and reduced the cytopathic effect (CPE) caused by virus infection. CPE was assessed by the CellTiter-Glo assay. The percent reduction in CPE was determined and EC_{50} was calculated as described above.

3. Results

3.1. Inhibition of Zika virus by MMPD

To evaluate its activity against ZIKV, MMPD was added to Huh7 hepatoma cells before virus infection. Culture supernatants were harvested on Day 2 for the measurement of viral RNA by qRT-PCR. MMPD inhibited ZIKV RNA replication with an EC_{50} of 0.6 ± 0.2 μM and EC_{90} of 1.0 ± 0.2 μM (average ± SD from 4 independent experiments). In the cytotoxicity assay CC_{50} was determined to be > 10 μM, which resulted in a selective index of > 17.

We next investigated whether MMPD could inhibit infectious ZIKV...
production. To better understand the effect of MMPD on host Vero cells during the longer dosing period, uninfected cells were treated for 7 days and cell proliferation was measured. As shown in Fig. 1A, a slight increase in MMPD cytotoxicity was observed over time from Day 1 to Day 7. The CC50 was 31 μM, 14 μM and 9.5 μM on Day 1, 3 and 7 respectively. In the presence of 100 μM of exogenous guanosine, the CC50 was > 50 μM, 47 μM and 36 μM on Day 1, 3 and 7 respectively (Fig. 1B). The mitigation of cytotoxicity by exogenous addition of guanosine suggests that the effect on cells is related to MMPD activity as an IMPDH inhibitor to reduce cellular GTP levels. For subsequent virus production assays, the highest concentration of MMPD tested was 10 μM, at or below the CC50 value on the longest dosing day in any experiment (Day 7).

Inhibition of ZIKV production by MMPD was demonstrated by treating infected Vero cells for 5 days and titering culture supernatants using plaque assays. When infected cells were treated with serially diluted MMPD (0.12–10 μM), reduction in ZIKV titer was observed with 3.3 and 10 μM MMPD (Table 1 and Fig. 2), but not at lower concentrations (data not shown). At these two higher concentrations, MMPD delayed the time of peak viral production from Day 2 to Day 3. Significant reduction in virus titer (P < 0.05) was observed early in the infection (Day 1 and 2) in a dose-dependent manner (~1 log and ~2 log reduction at 3.3 μM and 10 μM respectively). Addition of 100 μM guanosine reversed the inhibition by MMPD, consistent with its mechanism of action as an IMPDH inhibitor (Fig. 2). RBV was used as the control compound. Moderate inhibition of ZIKV was observed with RBV early in the infection, with 0.8 ± 0.3 and 1.0 ± 0.4 log reduction in virus titer on Day 1 and 2 respectively at 100 μM. Addition of guanosine reversed RBV inhibition (data not shown). In contrast, T-705 (favipiravir), a nucleoside inhibitor of influenza, Ebola and other viruses (Furuta et al., 2013; Westover et al., 2016), had little effect (<0.5 log reduction) on ZIKV at up to 100 μM (data not shown). The CC50 of RBV and T-705 was > 100 μM in the 7-day cytotoxicity assay. In a recent study, T-705 showed modest activity against ZIKV with an EC50 of ~20 μM (Zmurko et al., 2016). The difference in T-705 activity in the two studies could be due to the different ZIKV strains used (FSS13025 vs. MR766), and/or due to the different levels of T-705 triphosphate, the active metabolite, generated in Vero cells with different lineages and passaging histories.

### Table 1

Reduction in Zika virus titer (log_{10} [pfu/mL]) by MMPD.

<table>
<thead>
<tr>
<th>Day</th>
<th>MMPD (10 μM)</th>
<th>MMPD (3.3 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−1.7 ± 0.2*</td>
<td>−1.1 ± 0.3*</td>
</tr>
<tr>
<td>2</td>
<td>−2.3 ± 0.3*</td>
<td>−1.1 ± 0.6*</td>
</tr>
<tr>
<td>3</td>
<td>−0.1 ± 0.2</td>
<td>0.6 ± 0.1*</td>
</tr>
<tr>
<td>5</td>
<td>0.7 ± 0.6</td>
<td>0 ± 0.1</td>
</tr>
</tbody>
</table>

*Indicates the change in virus titer that is statistically significant relative to untreated infection control by unpaired t-test (P < 0.05).

Presented as average ± SD from 3 independent experiments.

Having demonstrated that MMPD inhibits ZIKV in vitro, we expanded the investigation to Ebola virus and several other emerging pathogens of global importance. Differences in viral replication kinetics were observed. The fastest growing virus appears to be ZIKV, with untreated infection control reaching peak virus titer on Day 2 (Fig. 2).
This was followed by Lassa virus and Junin virus, each peaking on Day 3, whereas the titer of Ebola virus kept increasing until Day 5 (Fig. 5A–C). MMPD was shown to inhibit virus production of Ebola virus (Fig. 5A), Lassa virus (Fig. 5B), and Junin virus (Fig. 5C) in a dose-dependent manner. In general, the largest effect of MMPD was seen on the day(s) when untreated infections approached peak virus titer. As summarized in Table 2, with Ebola virus, significant reductions (P < 0.05) in virus titer were observed on Day 3 and Day 5 at 3.3 μM (0.9–1 log reduction) and 10 μM (1.3–1.5 log reduction) of MMPD. For Lassa virus, inhibition of virus titer (0.7–1.6 log reduction) was maintained up to Day 7 at 10 μM MMPD, but little inhibition was seen at 3.3 μM MMPD (P > 0.05 from Day 3–7). More pronounced inhibition was observed with Junin virus, with 2.3 log reduction in virus titer at 10 μM and 1.5 log reduction at 3.3 μM MMPD on Day 3, although the virus titer rebounded on Day 7. For Chikungunya virus, the activity of MMPD was measured using a 3-day cytoprotection assay, resulting in an EC50 of 1.8 ± 1.0 μM (Fig. 5D). Cytotoxicity was measured in parallel with a CC50 of 27 ± 3 μM. Addition of 100 μM guanosine reversed the inhibition by MMPD on all viruses tested in this study (Fig. 5D and data not shown).

We next investigated the combination of MMPD with other antiviral agents in the virus production assay. In the combination experiments against Lassa virus and Junin virus, MMPD (3.3 μM and 10 μM) was co-dosed with RBV at 33 μM and 100 μM. In the combination experiments against Ebola virus, MMPD (3.3 μM and 10 μM) was co-dosed with T-705 at 33 μM and 100 μM. Fig. 6 shows the virus titer following combination treatment on the day when untreated infections reached peak virus titer (Day 3 for Lassa and Junin viruses, Day 5 for Ebola virus), and the numeric reduction in virus titer relative to untreated control can be found in Supplementary Table 2. Reduction in virus production was significantly enhanced (P < 0.05) by combination treatment compared with single agents (with the exception of one combination in the Ebola group). The maximum reduction in virus production was ~3 log for Lassa and Ebola viruses; the most significant enhancement was observed with Junin virus, where up to 5 log reduction in virus titer was observed (Supplementary Table 2). As previously shown in Fig. 5, addition of RBV or T-705 to MMPD did not significantly change cell viability (P > 0.05) when compared with MMPD single treatment, providing evidence that the enhanced inhibition on virus production in MMPD and RBV or T-705 combination treatment is specific to the antiviral activity and not solely due to the effect on cell viability.

4. Discussion

Until recently, ZIKV was an obscure member of the Flaviviridae family. Relatively little effort was undertaken to study its replication cycle or to develop prevention and treatment measures. The recent outbreaks of ZIKV and infection-associated fetal death and neurological disorders, such as microcephaly in newborns, have caused grave concerns for public health and highlighted the urgent need for antiviral interventions (Haug et al., 2016; Zanluca et al., 2016). One issue with ZIKV management is the challenge of rapid and accurate diagnosis as it can co-circulate with other important viral pathogens and share certain early clinical symptoms (Roth et al., 2014; Tilak et al., 2016). As a strategy to combat emerging viral threats, broad-spectrum antivirals targeting host factors or direct antiviral agents capable of inhibiting multiple viruses have become increasingly desirable (Bekerman and Einav, 2015). Several compounds have shown cross activities against ZIKV and other viral pathogens: BCX4430, a broad-spectrum adenosine analog (Julander et al., 2017); sofosbuvir, a nucleotide inhibitor of HCV (Bullard-Feibelman et al., 2017); duramycin, which also blocks infection of dengue, Ebola, and West Nile viruses (Tabata et al., 2016); as well as inhibitors of polyamine biosynthesis which have shown activity against ZIKV and diverse RNA viruses (Mounce et al., 2016).

MMPD is an IMPDH inhibitor that had been in development for the treatment of HCV. It was reported to inhibit HCV in cultured cells with an EC50 of 0.39 μM (Marcellin et al., 2007) and to have activity against a variety of DNA and RNA viruses in vitro (Markland et al., 2000). In HCV patients, MMPD was well-tolerated when combined with pegylated interferon alpha 2a and RBV for up to 48 weeks in phase 2a and 2b studies (Marcellin et al., 2007; Rustgi et al., 2009).

Our studies have expanded these previous findings and shown that MMPD inhibits ZIKV RNA replication in cell culture with an EC50 of 0.6 μM, similar to its in vitro potency against HCV. Furthermore, we demonstrated that MMPD is an inhibitor of several viral pathogens of global importance, such as Ebola, Lassa, Chikungunya and Junin...
viruses, raising the possibility of using MMPD as the backbone of prophylactic or therapeutic regimens to control emerging viral outbreaks. It is noted that combination of MMPD with another antiviral such as RBV or T-705 showed enhanced suppression of viral replication in virus production assays, although additional in vitro studies are required to further define the positive interaction (e.g. synergistic or additive) as well as the mechanism underlining the cooperation between MMPD and other antiviral drugs. For example, multiple enzymatic targets have been proposed for RBV in addition to IMPDH, among which are inhibition of incorporation by viral RNA polymerase and inhibition of viral mRNA capping by guanylyltransferase (Markland et al., 2000 and references therein). It would be interesting to evaluate whether MMPD would have any effect on these activities of RBV. Furthermore, the benefit of combination treatment should also be assessed in animal

Table 2
Reduction in virus titer (log10 [pfu/mL]) in EBOV, LASV and JUNV by MMPD. 

<table>
<thead>
<tr>
<th>Day</th>
<th>EBOV</th>
<th>LASV</th>
<th>JUNV</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.1 ± 0.1</td>
<td>-1.6 ± 0.4</td>
<td>-1.8 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>-1.3 ± 0.4</td>
<td>-0.7 ± 0.2</td>
<td>-2.3 ± 0.7</td>
</tr>
<tr>
<td>5</td>
<td>-1.5 ± 0.4</td>
<td>-0.7 ± 0.5</td>
<td>-1.8 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>-1.9 ± 1.3</td>
<td>-0.9 ± 0.4</td>
<td>-0.2 ± 0.4</td>
</tr>
</tbody>
</table>

*Indicates the change in virus titer that is statistically significant relative to untreated infection control by unpaired t-test (P < 0.05).

EBOV: Ebola virus; LASV: Lassa virus; JUNV: Junin virus.

a Presented as average ± SD from 3 to 4 independent experiments.
models. Clinically, RBV is the primary antiviral therapeutic for Lassa fever and has been used to treat Junin virus infections (Enria et al., 2008; Bausch et al., 2010; Vela, 2012). T-705 has been shown to improve survival and reduce viral load in Ebola infected patients (Bai et al., 2016; Sissoko et al., 2016). It would be important to explore whether the treatment of these viral infections can be improved by combining MMPD with RBV or T-705.

As with most host-targeting therapies, the window between antiviral activity and effect on host cells is of critical importance. MMPD has shown a good selective index in our experiments (SI > 17 against ZIKV). Additional evidence to support the specificity of MMPD as an antiviral agent is obtained by examining the kinetics of MMPD inhibition on virus production, which occurs early in the treatment (e.g. Day 2 and Day 3), and the kinetics of its effect on cell viability, which modestly yet progressively worsens with treatment time (up to Day 7).

In fact, the virus titer of treated groups often ‘catches up’ with that of untreated control late in the treatment when cell viability is the lowest. The mechanism of the transient nature of the antiviral activity is not yet understood; one possibility is that as infection proceeds, replicating viruses shut off host nucleic acid synthesis and reduce the demand on nucleotide pools to favor viral RNA replication at suboptimal GTP levels, thereby alleviating the inhibitory effect of MMPD. Nevertheless, the uncoupling of MMPD inhibition on virus titer and its effect on cell viability argues against the interpretation that the observed reduction in virus production is solely due to MMPD killing host cells. Future in vivo animal efficacy and toxicology studies are necessary to better determine the dose and therapeutic window of MMPD. Although MMPD, an inhibitor of an essential host enzyme with risks of potential clinical toxicity, may not be suitable for special populations such as pregnant women, MMPD can still play a useful role in controlling Zika infections. For example, it may be used to treat Zika-infected men to prevent sexual transmission to women of childbearing age. The availability of human PK and safety data from HCV trials could expedite the clinical development of MMPD. In summary, our results suggest that further studies are warranted in order to develop MMPD as a therapeutic for the treatment of ZIKV infection and other viral outbreaks.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2017.11.004

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Johnson, E.L., McDonald, C.E., Ma, H., O’Neal, J.T., 2016. The mechanisms of the transient nature of the antiviral activity is not yet understood; one possibility is that as infection proceeds, replicating viruses shut off host nucleic acid synthesis and reduce the demand on nucleotide pools to favor viral RNA replication at suboptimal GTP levels, thereby alleviating the inhibitory effect of MMPD. Nevertheless, the uncoupling of MMPD inhibition on virus titer and its effect on cell viability argues against the interpretation that the observed reduction in virus production is solely due to MMPD killing host cells. Future in vivo animal efficacy and toxicology studies are necessary to better determine the dose and therapeutic window of MMPD. Although MMPD, an inhibitor of an essential host enzyme with risks of potential clinical toxicity, may not be suitable for special populations such as pregnant women, MMPD can still play a useful role in controlling Zika infections. For example, it may be used to treat Zika-infected men to prevent sexual transmission to women of childbearing age. The availability of human PK and safety data from HCV trials could expedite the clinical development of MMPD. In summary, our results suggest that further studies are warranted in order to develop MMPD as a therapeutic for the treatment of ZIKV infection and other viral outbreaks.

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