**In vitro and in vivo efficacy of Molnupiravir against Zika virus infections**

Zhuang Wang, Shaokang Yang, Qingsong Dai, Xiaojia Guo, Yuexiang Li, Wei Li, Xiaotong Yang, Jingjing Yang, Xintong Yan, Huimin Tao, Chongda Luo, Song Li, Xingjuan Chen, Ruiyuan Cao, Song Li.  

EIDD-2801 showed favorable in vivo antiviral efficacy in a ferret influenza infection model (Toots et al., 2019). Wang et al. reported that EIDD-1931 significantly inhibited Crimean-Congo hemorrhagic fever virus (CCHFV) infection in Vero E6 cells (EC$_{50}$ = 5.19 ± 1.43 μmol/L, SI = 46.72). However, neither EIDD-1903 nor EIDD-2801 showed any protective effect in CCHFV-infected mice (Wang et al., 2022a). Another recent study found that EIDD-1931 was effective against Ebola virus challenge with 92%–100% protection in a BALB/c mouse model (Bluemling et al., 2022). Moreover, EIDD-2081 treatment inhibited viral replication in the lungs of hamsters infected with SARS-CoV-2 variants of concern and, in particular, in the respiratory tract of hamsters infected with the coronavirus Omicron variant (Rosenke et al., 2022). Currently, Molnupiravir is approved for the oral treatment of SARS-CoV-2 infections in the United States, United Kingdom, and China. It is important to systematically evaluate the in vitro and in vivo antiviral efficacy against other viruses with pandemic potential including ZIKV.

Firstly, we tested the antiviral activities of EIDD-2801 and EIDD-1931 by performing cytopathic effect (CPE) protection assay in baby hamster kidney (BHK) cells using a variety of flavivirus strains (Yang et al., 2022), including ZIKV, dengue virus (DENV), yellow fever virus (YFV), and Japanese encephalitis virus (JEV). As showed in Table 1, EIDD-2801 and EIDD-1931 exhibited broad-spectrum anti-flavivirus effects in BHK cells under non-cytotoxic conditions, and EIDD-1931 showed better efficacy against ZIKV, DENV, and YFV at lower concentrations.

Next, we further evaluated the anti-ZIKV activities of EIDD-2801 and EIDD-1931 in different cell lines (Fig. 1B and C). BHK and HuH7 cell lines were incubated with gradient-diluted compounds and ZIKV viromes (5.62 × 10^4 TCID$_{50}$ of viral stock was diluted to a 100 TCID$_{50}$ working solution) until cytopathic effect protection was observed. The results showed that the half-maximal inhibitory concentration (IC$_{50}$) values of
Table 1

<table>
<thead>
<tr>
<th>Flavivirus</th>
<th>EIDD-2801 IC₅₀ (μmol/L)</th>
<th>EIDD-1931 IC₅₀ (μmol/L)</th>
<th>EIDD-2801 CC₅₀ (μmol/L)</th>
<th>EIDD-1931 CC₅₀ (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIKV</td>
<td>27.33 ± 2.67</td>
<td>&gt; 200</td>
<td>10.31 ± 0.11</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>DENV</td>
<td>&gt; 66.67</td>
<td>&gt; 200</td>
<td>14.65 ± 0.16</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>YFV</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>24.14 ± 17.49</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>JEV</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 66.67</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

IC₅₀ and CC₅₀ values were determined in three independent experiments. The data were presented as the mean ± standard deviation (SD). Abbreviations: Zika virus (ZIKV), dengue virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), half-maximal inhibitory concentration (IC₅₀), and 50% cytotoxic concentration (CC₅₀).

EIDD-2801 in BHK and HuH7 cells were 27.33 ± 2.67 μmol/L and 78.35 ± 1.48 μmol/L, respectively. For EIDD-1931, the IC₅₀ values were 10.31 ± 0.11 μmol/L and 14.91 ± 0.73 μmol/L, respectively. Besides, both compounds showed acceptable in vitro safety profiles.

We then used EIDD-1931, the in vivo active form of EIDD-2801, to further explore the antiviral potential (Toots et al., 2019). First, we performed qRT-PCR and viral plaque forming unit (PFU) assays. BHK cells were inoculated with ZIKV (MOI = 0.05) in the presence of the gradient-diluted EIDD-1931. After 72 hours, the supernatant was collected to detect infectious virus particles by qRT-PCR and Western blotting, respectively. As expected, EIDD-1931 significantly inhibited the production of progeny virus particles (Fig. 1D) and the replication of viral RNA (Fig. 1E). As shown in Fig. 1F, 50 and 25 μmol/L EIDD-1931 treatment reduced viral structural (E protein) and non-structural protein (NS5 protein) expression, which was consistent with the results of the viral RNA and PFU assays. Furthermore, an immunofluorescence assay was performed to visualize the inhibition of ZIKV by diluted EIDD-1931 in BHK cells. The result showed that EIDD-1931 inhibited viral protein production in a dose-dependent manner (Fig. 1G). The collective findings indicate the favorable in vitro anti-ZIKV activities of EIDD-1931.

A fatal one-day-old ICR suckling mouse ZIKV challenge model (Wang et al., 2022b) was used to evaluate the anti-ZIKV efficacy of EIDD-2801 and EIDD-1931 in vivo. The suckling mice were randomly grouped, followed by intraperitoneal (i.p.) injection with 1.2 × 10⁸ PFU of ZIKV per mouse. The mice were then i.p. injected with 100, 50 or 25 mg/kg EIDD-2801, 50 or 25 mg/kg EIDD-1931, or saline (control group) daily for nine consecutive days (Li et al., 2022). Treatment with 100 mg/kg EIDD-2801 protected 80.0% of the mice from lethal challenge of ZIKV (Fig. 1H). EIDD-1931 also showed dose-dependent protection. An administration with 50 mg/kg EIDD-1931 protected 55.6% of mice from death. The body weight of mice in the EIDD-1931 and EIDD-2801 treated groups increased, while decreased body weight was observed in the saline-treated group (Fig. 1H and I). To further characterize the in vivo antiviral effects of EIDD-2801 and EIDD-1931, the viral RNA in blood from ZIKV-infected mice was measured using the qRT-PCR assay at day four post infection. As shown in Fig. 1J, ZIKV RNA levels were significantly reduced in the 100 mg/kg EIDD-2801 treated and 50 mg/kg EIDD-1931 treated groups. In addition, hematoxylin and eosin staining of brain or liver tissue collected at day four post infection showed an alleviated tissue damage in the drug treatment groups (Fig. 1K and L) (Wang et al., 2018). Taken together, the above results indicated that EIDD-2801 and EIDD-1931 possess promising in vivo antiviral activities.

We next conducted a time-of-drug-addition assay to explore the stage of action of Molnupiravir. As shown in the schematic diagram (Fig. 1M), with ZIKV added at 0–2 h, EIDD-1931 was added at different time points corresponding to the different stages of the viral life cycle (Yan et al., 2022). In Fig. 1N, both EIDD-1931 and NITD008 (a positive control drug acting on the viral RNA replication stage), reduced viral RNA replication at stage III/IV, suggesting that EIDD-1931 might act on the post-entry stage of ZIKV. The inhibitory effect of EIDD-1931 was also evaluated using a BHK cell line possessing a ZIKV replicon (BHK-ZIKV replicon cells) (Li et al., 2018). As expected, EIDD-1931 inhibited the replication of ZIKV replicon (IC₅₀ = 24.38 ± 0.73 μmol/L, SI > 4.10) as did the positive control drug 2’-CMA (IC₅₀ = 5.64 ± 0.81 μmol/L, SI = 4.32) (Fig. 1O and P). These results suggest that the anti-ZIKV activities of EIDD-1931 and EIDD-2801 involve the genome replication stage of ZIKV.

ZIKV is a highly focused flavivirus with global pandemic potential (Sargent et al., 2022). In this study, we evaluate the anti-ZIKV activity of Molnupiravir and expand its antiviral spectrum. The findings also demonstrate that Molnupiravir blocks ZIKV infection by inhibiting viral genome replication throughout the viral life cycle, with potent in vitro and in vivo efficacy. As a nucleoside analog, it probably acts by interfering with viral RdRp and induces genome mutation or chain termination. However, the specific mode of Molnupiravir needs to be further verified using the crystal structure of ZIKV RdRp. In this study, we provide experimental evidence that Molnupiravir is a promising antiviral drug candidate for flaviviruses including ZIKV. Considering that Molnupiravir is an approved novel antiviral drug that can be orally administered, it is of importance to conduct further clinical studies to evaluate its clinical efficacy against flavivirus infections.
Footnotes

This research was funded by the National Natural Science Foundation of China (NSFC, grants 81773631, to R.C.; grants 81900402, to X.C.); the National Science and Technology Major Projects for “Major New Drugs Innovation and Development”, China (2018ZX09711003, to W.Z.). The authors declare no conflict of interest. All animal experiments performed in this study are approved by the IACUC (Institutional Animal Care and Use Committee) of Beijing Institute of Pharmacology and Toxicology. All works with infectious virus were carried out in Biosafety Level 2 (BSL-2) or Animal Biosafety Level 2 laboratory (ABSL-2).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.05.011.

References


