



Short Communication

Remdesivir triphosphate can efficiently inhibit the RNA-dependent RNA polymerase from various flaviviruses

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ABSTRACT

Remdesivir was shown to inhibit RNA-dependent RNA-polymerases (RdRp) from distinct viral families such as from *Filoviridae* (Ebola) and *Coronaviridae* (SARS-CoV, SARS-CoV-2, MERS). In this study, we tested the ability of remdesivir to inhibit RdRps from the *Flaviviridae* family. Instead of remdesivir, we used the active species that is produced in cells from remdesivir, the appropriate triphosphate, which could be directly tested *in vitro* using recombinant flaviviral polymerases. Our results show that remdesivir can efficiently inhibit RdRps from viruses causing severe illnesses such as Yellow fever, West Nile fever, Japanese and Tick-borne encephalitis, Zika and Dengue. Taken together, this study demonstrates that remdesivir or its derivatives have the potential to become a broad-spectrum antiviral agent effective against many RNA viruses.

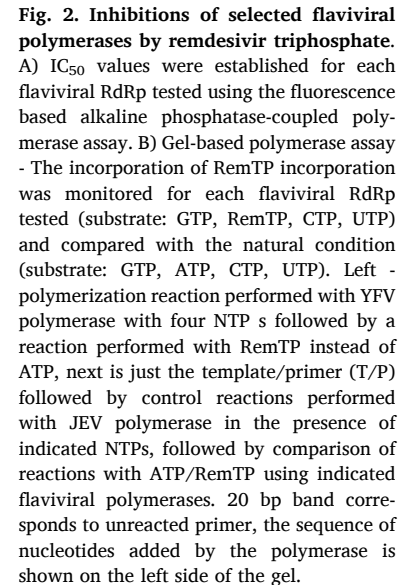
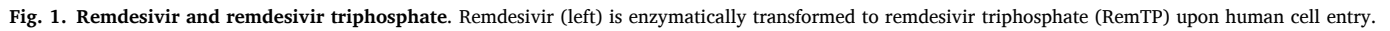
Flaviviruses are arthropod-borne single-stranded positive sense RNA viruses (+RNA viruses) that cause numerous human diseases. These pathogens are usually transmitted by mosquitoes or ticks. A genome of flaviviruses is about 11 kb long and it has only one open reading frame, which is transcribed into a single polyprotein, that is subsequently cut in three structural and seven nonstructural viral proteins either by viral or host proteases (Kok, 2016; Weaver and Reisen, 2010). Among the nonstructural proteins (NS), NS5 has a unique position since it has two very important functions connected to two distinct protein domains. It has RNA-dependent RNA polymerase (RdRp) functionality, which is connected to the C-terminus of the protein. The N-terminal domain acts as viral RNA methyltransferase. While RdRp is essential for copying +RNA to and from -RNA, the methyltransferase is responsible for RNA cap creation, which is indispensable for normal function of viral RNA and shields it from innate immunity (Dong et al., 2014; Garcia-Blanco et al., 2016; Lescar et al., 2012; Lim et al., 2015; Ray et al., 2006; Sampath and Padmanabhan, 2009). Importantly, NS5 protein possesses highly conserved drug targets shared among flaviviruses (Dubankova and Boura, 2019).

Among the numerous other flaviviruses, special attention has been paid to viruses that caused larger outbreaks in recent years. First, Dengue virus (DENV) causes approximately 390 million infections each year, of which nearly 100 million have clinical manifestations, which

makes it the most common flavivirus infection globally (Behnam et al., 2016; Chan et al., 2015; Lescar et al., 2018). West Nile virus (WNV) affects numerous patients in North America, including USA, every year. Although the occurrence of WNV neuroinvasive disease is rather rare its fatality rate is around 10% (Benjelloun et al., 2016; Farrar, 2013; Kramer et al., 2007). Zika virus caused a significant epidemic in 2015 and 2016, which was associated with a significant increase in concerns over flavivirus diseases and a significant revival of research into new anti-flavivirus substances (Paixao et al., 2016; Weaver et al., 2016). Yellow fever virus (YFV) and Japanese encephalitis virus (JEV) have also continued to be a significant health problem in South and Central America and Africa, and in Asia and Oceania, respectively, despite the availability of an effective vaccine. Similarly, tick-borne encephalitis virus (TBEV) causes numerous infection in Czechia, Germany, Austria and other central European countries as well as in Russia during the period of activity of locally occurring ticks of the genus *Ixodes* (Chrdle et al., 2016).

Contemporary antiviral therapies are often based on nucleoside and nucleotide derivatives, which target the viral polymerase, the central enzyme of the virus. Therefore, compounds targeting RdRp are expected to also be the backbone of anti-flavivirus therapy for numerous diseases caused by members of this genus (Eyer et al., 2016a, 2016b, 2017, 2018; Hercík et al., 2017; Malet et al., 2008; Sebera et al., 2018).

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We aimed to test remdesivir directly in a polymerase assay *in vitro*. The prodrug form of remdesivir is not suitable because the active form is remdesivir triphosphate (RemTP) unveiled enzymatically from remdesivir once remdesivir is inside mammalian cells (Warren et al., 2016). We have used a previously published protocol to synthesize RemTP chemically (Cho et al., 2012) and obtained sufficient amounts for our experiments. Next, we performed the polymerase assay using the alkaline phosphatase-coupled polymerase assay (FAPA) (Niyomrattanakit et al., 2011) as detailed in SI. The assay revealed that remdesivir inhibits all the tested flaviviral polymerases with an IC₅₀ in the range 0.2–2.2 μM. The best IC₅₀ value (0.26 ± 0.03 μM) was observed for the YFV polymerase. All the other polymerases tested (DENV3, TBEV, JEV, WNV, ZIKV) were inhibited similarly with IC₅₀ ranging from 1.3 to 2.2 μM (Fig. 2A). Next we used a gel-based RNA polymerization assay to gain

deeper insight into the remdesivir mechanism of action. We expected to observe the delayed termination of the transcription mechanism of action that was reported for Ebola and coronavirus polymerases (Gordon et al., 2020a, Gordon et al., 2020b; Tchesnokov et al., 2019). However, all six flaviviral polymerases tested were able to incorporate several (five) remdesivir molecules into the newly synthesized RNA chain and finish the primer elongation reaction (Fig. 2B). We also noted that the 20 bp band corresponding to the primer disappeared in the case of JEV and ZIKV polymerase, we attribute it to endonuclease activity of these polymerases under these reactions conditions (3 mM MnCl₂), which was not observed under the same conditions but in 1 mM MnCl₂ (SI Fig. 2), however, we cannot exclude the possibility that JEV and ZIKV enzymes were contaminated by an exonuclease that is active only in 3 mM MnCl₂. Our results confirm and explain the observed effect of remdesivir on the TBEV in cell culture-based experiments (Lo et al., 2017) albeit they suggest that the mechanism of action, in case of flaviviruses, cannot be ascribed to delayed chain termination.

Remdesivir is known to be a potent inhibitor of Ebola RdRp (Tchesnokov et al., 2019) and several coronaviral RdRps (Brown et al., 2019; Gordon et al., 2020a, Gordon et al., 2020b). In this study, we show that remdesivir also efficiently inhibits flaviviral RdRps using pure recombinant RdRps and RemTP. The IC₅₀s range from hundreds of nanomolar to single digit micromolar suggesting that flaviviral polymerases are a weaker target for remdesivir than the coronaviral polymerases where the reported IC₅₀ values are in the tens of nanomoles (Gordon et al., 2020a). Taken together, our data show that remdesivir is a single-digit-micromolar inhibitor of flaviviral RdRps and suggest that its structure, which was originally designed to combat Ebola, could be optimized to combat multiple, if not all, diseases caused by flaviviruses.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.antiviral.2020.104899>.

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