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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 anthropod-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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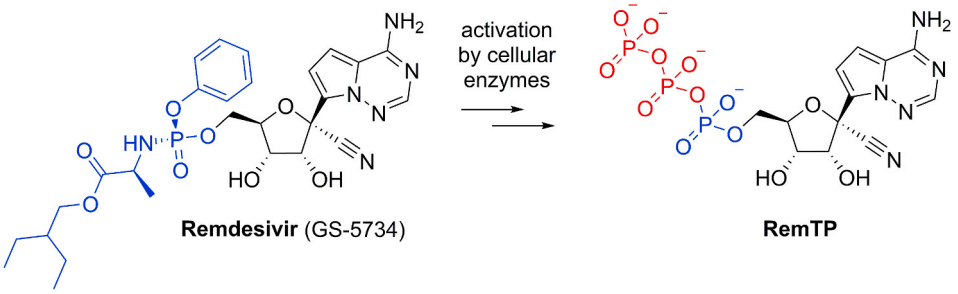

Fig. 1. Remdesivir and remdesivir triphosphate. Remdesivir (left) is enzymatically transformed to remdesivir triphosphate (RemTP) upon human cell entry.

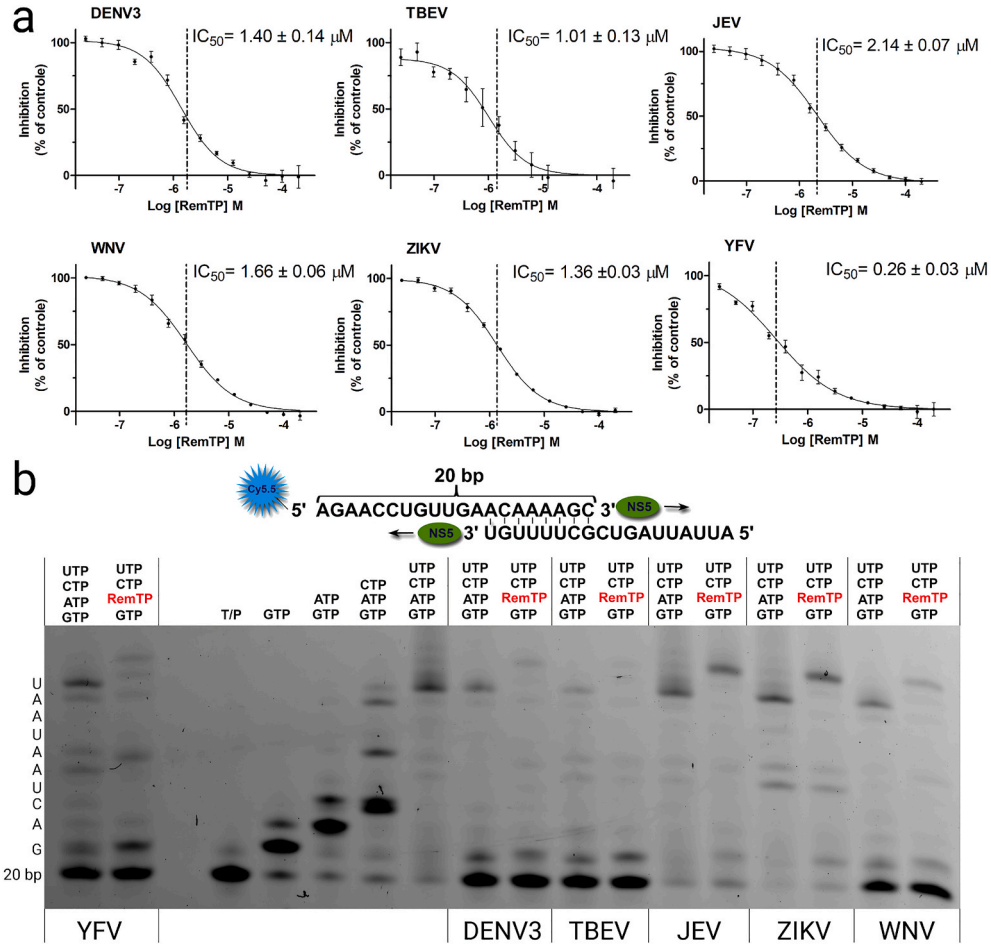

Fig. 2. Inhibitions of selected flaviviral polymerases by remdesivir triphosphate. A) IC_{50} values were established for each flaviviral RdRp tested using the fluorescence based alkaline phosphatase-coupled polymerase assay. B) Gel-based polymerase assay - The incorporation of RemTP incorporation was monitored for each flaviviral RdRp tested (substrate: GTP, RemTP, CTP, UTP) and compared with the natural condition (substrate: GTP, ATP, CTP, UTP). Left - polymerization reaction performed with YFV polymerase with four NTP s followed by a reaction performed with RemTP instead of ATP, next is just the template/primer (T/P) followed by control reactions performed with JEV polymerase in the presence of indicated NTPs, followed by comparison of reactions with ATP/RemTP using indicated flaviviral polymerases. 20 bp band corresponds to unreacted primer, the sequence of nucleotides added by the polymerase is shown on the left side of the gel.

Remdesivir, a monophosphate prodrug of a modified adenosine, has recently received a lot of attention due to its applicability for treatment of coronavirus infection caused by severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) (Brown et al., 2019; de Wit et al., 2020; Sheahan et al., 2017). The compound was originally developed as a potential treatment for the Ebola virus and it also proved to be active against numerous other RNA viruses including Filo-, Pneumo-, and Paramyxoviruses (Lo et al., 2017; Siegel et al., 2017; Warren et al., 2016).

Since remdesivir was reported to inhibit distinct viral polymerases we aimed to test its ability to inhibit various flaviviral polymerases. Expression plasmids encoding NS5s from TBEV, DENV3, JEV, and WNV were ordered from the European Virus Archive (EVAg), expression plasmids encoding the ZIKV and YFV NS5s were described previously (Dubankova and Boura, 2019; Hercik et al., 2017). We have expressed all the NS5s proteins in *E. coli* and purified them using our previous protocol developed for ZIKV NS5 protein (Hercik et al., 2017) as detailed in Supplementary Information (SI Fig. 1).

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 synthetize 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 IC_{50} in the range 0.2–2.2 μM. The best IC_{50} 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 IC_{50} 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 $MnCl_2$), which was not observed under the same conditions but in 1 mM $MnCl_2$ (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 $MnCl_2$. 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 IC_{50}s 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 IC_{50} 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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