

SHORT COMMUNICATION

Remdesivir potently inhibits carboxylesterase-2 through covalent modifications: signifying strong drug-drug interactions

Yue Shen, William Eades, Bingfang Yan 

Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA

Keywords

COVID-19, remdesivir, carboxylesterases, drug safety

Received 15 November 2020;
revised 23 December 2020;
accepted 23 December 2020

Correspondence

Bingfang Yan, Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH 45229, USA.
Email: yanbg@uc.edu

FUNDING

This work was supported by National Institutes of Health Grants R01 EB018748 and R21AI153031-01 (Yan B).

ABSTRACT

Remdesivir was recently approved to treat COVID-19. While this antiviral agent delivers clinical benefits, several safety concerns in many cases have been raised. This study reports that remdesivir at nanomolar concentrations inhibits carboxylesterase-2 (CES2) through covalent modifications. CES2 is a major drug-metabolizing enzyme. The combination of high potency with irreversible inhibition concludes that cautions must be exercised when remdesivir is used along with drugs hydrolyzed by CES2.

The pandemic of coronavirus disease 2019 (COVID-19) has become a health crisis with the global death toll passing one million. So far, there are limited options to treat COVID-19. Remdesivir was granted emergency use authorization based on promising clinical benefits.^{1–3} Even with remdesivir, the rate of serious adverse events and mortality was high.^{1–3} The precise mechanisms remain unclear. Nevertheless, COVID-19 patients frequently receive multiple drugs and remdesivir requires metabolism for its therapeutic activity^{1–3}; therefore, metabolism-based interactions are likely contributing factors.

Remdesivir is an ester prodrug and undergoes hydrolysis, most likely by carboxylesterase-1 (CES1). In addition, remdesivir has a core-structure alanine (boxed in Figure 1a) as seen with orlistat and sofosbuvir, two covalent inhibitors of CES2.^{4,5} We therefore hypothesized that remdesivir irreversibly inhibits CES2. To test this hypothesis, human liver microsomes pooled from 23 donors, incubated with remdesivir (0, 0.2 and 1 $\mu\text{mol/L}$), electrophoretically separated and tested for the remaining hydrolytic activity in the gel. Electrophoresis removed unbound but not covalently bound inhibitor, establishing an involvement of

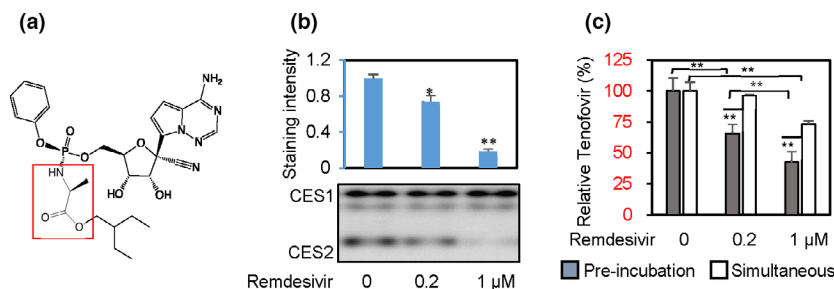


Figure 1 Irreversible inhibition of CES2 by remdesivir, and its effect on the hydrolysis of tenofovir disoproxil fumarate (TDF). (a) Structure of remdesivir (alanine boxed). (b) Native gel electrophoresis stained for hydrolytic activity. Microsomes pooled from 23 individual donors (5 μ g) were incubated with remdesivir at 0, 0.2 or 1.0 μ mol/L and subjected to native gel electrophoresis stained for esterase activity with 4-methylumbelliferylacetate, and the staining intensity was captured by Bio-Rad ChemoDoc Imager. (c) Inhibition of liver microsomal hydrolysis of TDF by remdesivir. *Pre-incubation format*: pooled liver microsomes (0.06 μ g/ μ L) were pre-incubated at 37°C for 120 min with remdesivir at 0, 0.2 or 1.0 μ mol/L followed by TDF at 10 μ mol/L. *Simultaneous format*: pooled liver microsomes (0.06 μ g/ μ L) were incubated at 37°C with TDF (10 μ mol/L) and remdesivir (0, 0.2 or 1.0 μ mol/L). For both formats, the reactions lasted for 5 min (after addition of TDF) and were terminated by two volumes of termination buffer (acetonitrile and methanol: 50:50) containing the internal standard tenofovir [adenine- 13 C(U)](438 ng/mL). The reaction mixtures were subjected to centrifugation at 4°C for 15 min to remove the proteins, and the supernatants were analysed for the formation of tenofovir at the mass transitions of m/z 288.2-176.1 and internal standard at 293.2-181.1 by liquid chromatography with tandem mass spectrometry (TSQ Fortis). The quantification was determined with the standard curve generated with tenofovir. All experiments were performed in triplicate. Single asterisk denotes statistical significance at $p < 0.05$ whereas double asterisk at $p < 0.01$.

covalent modifications in the inhibition. As shown in Figure 1b, remdesivir at 0.2 μ mol/L significantly inhibited CES2. At 1 μ mol/L, remdesivir inhibited CES2 by as much as 81%. In a striking contrast, no inhibition was detected on CES1, pointing to the high specificity of the inhibition.

To shed light on the significance of CES2 covalent inhibition, pooled microsomes were incubated with remdesivir (0, 0.2 and 1 μ mol/L) and tested for reduced hydrolysis of tenofovir disoproxil fumarate (TDF). TDF is a widely used antiviral agent primarily hydrolyzed by CES2⁵ with a potential of being used for COVID-19. The incubations were performed in two formats: remdesivir and TDF were added at the same time (simultaneous), or remdesivir was pre-incubated followed by addition of TDF. The hydrolysis of TDF was monitored for the formation of tenofovir (hydrolytic metabolite) by liquid chromatography with tandem mass spectrometry. As shown in Figure 1c, simultaneous incubation at 0.2 μ mol/L decreased TDF hydrolysis by 4% whereas by 27% at 1 μ mol/L ($p < 0.01$). Pre-incubation caused a greater inhibition: 0.2 μ mol/L decreased the hydrolysis by 34% and 1 μ mol/L by 58% ($p < 0.01$ for both). The greater inhibition of pre- over simultaneous incubation suggested that the covalent inhibition (i.e. pre-incubation) caused the primary concern.

Remdesivir exhibited clinical benefits for COVID-19 patients; however, serious safety concerns were raised.¹⁻³ These concerns are broad and the liver toxicity is a major one.¹⁻³ Carboxylesterases hydrolyze a drug into two parts with profound pharmacological and toxicological significance. This study reports remdesivir as a potent and covalent CES2 inhibitor. Such combination exerts efficacious and sustained inhibitory activity towards CES2, a major hydrolase known to metabolize many drugs and toxicants. The finding provides a mechanistic explanation to the observed high rate of serious adverse events and mortality with the use of remdesivir.¹⁻³ As a result, caution on the use of remdesivir, in spite of promising clinical benefits, must be exercised for remdesivir-based drug interactions with serious clinical concerns.

REFERENCES

- 1 Beigel JH, Tomashek KM, Dodd LE et al. Remdesivir for the treatment of Covid-19 - final report. *N Engl J Med*. 2020;383:1813-1826.
- 2 Wang Y, Zhang D, Du G et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2020;395:1569-1578.

- 3 Grein J, Ohmagari N, Shin D et al. Compassionate use of remdesivir for patients with severe Covid-19. *N Engl J Med*. 2020;**382**:2327-2336.
- 4 Xiao D, Shi D, Yang D, Barthel B, Koch TH, Yan B. Carboxylesterase-2 is a highly sensitive target of the antiobesity agent orlistat with profound implications in the activation of anticancer prodrugs. *Biochem Pharmacol*. 2013;**85**:439-447.
- 5 Shen Y, Yan B. Covalent inhibition of carboxylesterase-2 by sofosbuvir and its effect on the hydrolytic activation of tenofovir disoproxil. *J Hepatol*. 2017;**66**: 660-661.