The *in vitro* antiviral activity of the anti-hepatitis C virus drugs daclatasvir and sofosbuvir against SARS-CoV-2

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Background

The SARS-CoV-2 pandemic continues to cause major global morbidity, mortality and economic burden. The public health urgency led to the study of clinically approved drugs as repurposed medicines to treat individuals with COVID-19. The current approaches have not proven successful to date. The understanding of preclinical activity, mechanism of action, pharmacokinetics and safety are critical to achieving clinical benefits from repurposing drugs. Daclatasvir (DCV) and sofosbuvir (SFV) are clinically approved antivirals against hepatitis C virus (HCV), with satisfactory safety profile. In the HCV replicative cycle, DCV and SFV target the viral enzymes NS5A-endowed with pleotropic activities which overlap with several proteins from SARS-CoV-2, and NS5B-HCV RNA polymerase that share homology with SARS-CoV-2`s. These similarities provide a basis to further study the activity of DCV and SFV against the new coronavirus.

Methods

Different cells types were infected with SARS-CoV-2 - Vero, Huh-7, Calu-3, primary human monocytes and NSCs derived from human iPS - to evaluate DCV and SFV antiviral activity. Culture supernatants were collected for virus titration (PFU/mL or qRT-PCR) and cytokine measurements. Cell death was evaluated by TUNEL staining. Cytotoxicity assays were performed in Vero, Huh-7 and Calu-3. Time-of-addition assays were executed with DCV and treatments started from 2h before to 18h after infection. Melting profiles were obtained by incubating SARS-CoV-2 RNA with DCV and Sybergreen in a Real-Time PCR. DCV-induced mutant viruses were generated by sequencing passages of SARS-CoV-2 in Vero with increasing concentrations of DCV. For *in silico* study, the structures of the active metabolite of SFV and DCV were docked into the crystal structure of the SARS-Cov-2 nsp12. PBPK model was generated to estimate DCV`s dose and schedule to maximize probability of success for COVID-19.

Results

DCV consistently inhibited the production of infectious SARS-CoV-2 in Vero, HuH-7 and Calu-3 cells, with potencies of 0.8, 0.6 and 1.1 µM, respectively. Although less potent than DCV, SFV and its nucleoside metabolite inhibited replication in Calu-3 cells. Moreover, SFV/DCV combination (1:0.15 ratio) inhibited SARS-CoV-2 with EC_{50} of 0.7:0.1 µM in Calu-3 cells. SFV and DCV prevented virus-induced neuronal apoptosis and release of cytokine storm-related inflammatory mediators by monocytes, respectively. Both drugs inhibited independent events during RNA synthesis and this was particularly the case for DCV, which also targeted secondary RNA structures in the SARS-CoV-2 genome. Concentrations required for partial DCV in vitro activity are achieved in plasma at Cmax after administration of the approved dose to humans.

Conclusions

Effective early antiviral interventions are urgently required for the SARS-CoV-2 pandemic. The presented data for two widely available anti-HCV drugs, particularly for DCV, provide a rational basis for further validation of these molecules for anti-SARS-CoV-2 interventions.