

Lack of Antiviral Activity of Darunavir against SARS-CoV-2

Sandra De Meyer PhD*, Denisa Bojkova MSc, Jindrich Cinatl PhD, Ellen Van Damme PhD, Christophe Buyck MEng, Marnix Van Look PhD, Brian Woodfall MD and Sandra Ciesek MD

¹Janssen Pharmaceutica NV, Beerse, Belgium (S. De Meyer, E. Van Damme, C. Buyck, M. Van Look)

²Janssen Biopharma Inc, South San Francisco, CA, USA (B. Woodfall)

³Institute of Medical Virology, University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany (D. Bojkova, J. Cinatl, S. Ciesek)

⁴German Center for Infection Research (DZIF), Frankfurt, Germany (S.Ciesek)

*Corresponding Author: sdmeyer@its.jnj.com

Abstract

Given the high need and the absence of specific antivirals for treatment of COVID-19 (the disease caused by severe acute respiratory syndrome-associated coronavirus-2 [SARS-CoV-2]), human immunodeficiency virus (HIV) protease inhibitors are being considered as therapeutic alternatives. Prezcobix/Rezolsta is a fixed-dose combination of 800 mg of the HIV protease inhibitor darunavir (DRV) and 150 mg cobicistat, a CYP3A4 inhibitor, which is indicated in combination with other antiretroviral agents for the treatment of HIV infection. There are currently no definitive data on the safety and efficacy of DRV/cobicistat for treatment of COVID-19. The *in vitro* antiviral activity of darunavir against a clinical isolate from a patient infected with SARS-CoV-2 was assessed. DRV showed no activity against SARS-CoV-2 at clinically relevant concentrations ($EC_{50} > 100 \mu\text{M}$). Remdesivir, used as a positive control, showed potent antiviral activity ($EC_{50} = 0.38 \mu\text{M}$). Overall, the data do not support the use of DRV for treatment of COVID-19.

Introduction

In December 2019, the severe acute respiratory syndrome-associated coronavirus disease-2019 (SARS-CoV-2; COVID-19) emerged in Wuhan, Hubei Province, China (1). The virus was subsequently identified as a coronavirus (CoV), in addition to SARS-CoV-1 and Middle East respiratory syndrome CoV (MERS-CoV) that passed from animals to humans where it can cause severe respiratory illness (2). As of March 2020, COVID-19 has spread around the world with the WHO declaring a global pandemic (3). Given the extent of the COVID-19 pandemic, there is an urgent need to identify potential treatments for the disease as well as to develop a vaccine.

As no specific antivirals for treatment of COVID-19 are available, one avenue of clinical interest is the use of human immunodeficiency virus (HIV) protease inhibitors (PIs) as a therapeutic intervention. The potential for HIV PIs as a treatment for COVID-19 is mainly based on limited virologic and clinical data on the HIV protease inhibitor lopinavir with low-dose ritonavir (as a pharmacoenhancer; LPV/r) in patients infected with severe acute respiratory syndrome related to a coronavirus (SARS-CoV) (4). After demonstrating the *in vitro* antiviral activity of LPV against SARS-CoV-1, the clinical response of patients with SARS to a combination of LPV/r and ribavirin was examined. Patients treated with LPV/r had lower rates of adverse clinical outcomes at day 21 following the onset of symptoms compared with historical controls (4). However, recent data in hospitalized adults with severe confirmed COVID-19 treated with LPV/r in addition to a standard care of ventilation, oxygen, vasopressor support, antibiotics and renal-replacement therapy showed that there was no significant improvement in time to clinical improvement or mortality at day 28 compared with the standard care (5).

The HIV PI darunavir with cobicistat as a pharmacoenhancer (DRV/c, 800/150 mg given orally once daily with food) in combination with other antiretroviral agents is approved for both treatment-naïve and -experienced patients with HIV-1 infection (6-7). The efficacy and safety

profile of boosted-DRV combination therapy is well-established in the HIV setting, based on phase III clinical studies as well as real-world evidence (8–11).

To date, no clear clinical evidence supports the use of DRV (boosted with either ritonavir or cobicistat) in viral diseases other than HIV.

In this paper, the antiviral activity of DRV against SARS-CoV-2 was investigated in an *in vitro* model at clinically relevant concentrations. When DRV/c is taken at the indicated once-daily dose, the median trough plasma concentration of DRV was 3.4 μM (1875 ng/mL). (6). This cell culture assay was shown to be suitable for antiviral assays. Productive viral infection takes place in this model with the number of SARS-CoV RNA molecules increasing continuously after infection, indicating that the virus undergoes full replicatory cycles (12). Remdesivir (GS-5734), a nucleotide analog initially developed for Ebola virus disease, has shown to inhibit SARS-CoV-2 replication *in vitro* with an EC_{50} equal to 0,770 μM (13) and was therefore used as a positive control.

Methods

Cell Culture and Virus Preparation

Human colon carcinoma cell line (Caco-2) cells (obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) were cultured in Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and containing penicillin (100 IU/mL) and streptomycin (100 µg/mL) in a 5% CO₂ atmosphere at 37°C. All culture reagents were purchased from Sigma (Hamburg, Germany).

SARS-CoV-2 was isolated from human samples and cultured in Caco-2 cells, as previously described (3). After one passage in Caco-2 cells, viral stocks were stored at –80°C prior to use.

Assessment of Antiviral Activity by Inhibition of Virus-Induced Cytopathogenic Effect

Confluent layers of Caco-2 cells were cultured at 37°C in a 5% CO₂ atmosphere for 72 hours on 96 multi-well plates (50,000 cells/well). Cells were challenged with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.01. Virus was added together with the compounds under investigation and incubated in MEM supplemented with 1% FBS.

DRV and remdesivir were synthesized at Johnson & Johnson. To assess *in vitro* antiviral activity, DRV and remdesivir, diluted in MEM without FBS, were added in 4-fold dilutions to a concentration range of 0.02 µM to 100 µM. Cells were then incubated for 48 hours before the cytopathogenic effect (CPE) was visually scored by two independent laboratory technicians. Evaluation of CPE was also done using an 3-(4,5-dimethyliazol-2-yl)-2,5-difeniltetrazolio (MTT; Sigma-Aldrich) method according to the manufacturer's instructions. Optic densities were measured at 560/620 nm in a Multiskan Reader MCC/340 Labsystems. Three independent experiments with triplicate measurements were performed. Evaluation of inhibition of CPE using

the MTT method was done for two of the three experiments. Data were analyzed by a four-parameter curve-fitting from a dose-response curve using GraphPad Prism (version 7.00) to calculate the EC₅₀ (concentration of the compound that inhibited 50% of the infection) based on visual CPE scoring or based on the MTT method.

Assessment of Cell Viability

To assess the effects of the compounds on Caco-2 cell viability, cell viability was measured in confluent cell layers treated with a range of compound concentrations in absence of virus using the Rotitest Vital (Roth) according to manufacturer's instructions, as previously described (12). All assays were performed three times independently in triplicate. From this the CC₅₀ (cytotoxic concentration of the compound that reduced cell viability to 50%) was calculated from a dose-response curve in GraphPad Prism (version 7.00) using four-parameter curve-fitting.

Selectivity Index

The selectivity index for each of the compounds was determined as the ratio of the CC₅₀ to the EC₅₀.

Modelling

In general, *in silico* docking can be a useful approach for identifying subsets of molecules for *in vitro* studies and can be used to explain *in vitro* observations on a structural level. The coordinates of the crystal structure of the main SARS-CoV-2 protease were retrieved from the PDB database (<https://www.rcsb.org/structure/6lu7>).

Preparing DRV and the protein for *in silico* molecular docking was performed with the software package MOE (Molecular Operating Environment 2019.01; Chemical Computing Group ULC, Montreal, QC, Canada, H3A 2R7, 2019). The force field used was AMBER ETH:10 with default

'quickprep' settings to prepare the protein complex. The original ligand was then removed.

General docking settings were then altered to have 50 initial placements.

Results

Assessment of *In Vitro* Antiviral Activity, Cytotoxicity and Selectivity

Remdesivir showed strong antiviral activity against SARS-CoV-2 with an EC₅₀ of 0.11 µM based on visual scoring of inhibition of CPE (Figure 1a). In the same experiments, DRV did not show any inhibition of SARS-CoV-2 induced CPE (Figure 1b; EC₅₀ >100 µM). Similar results were obtained using the MTT method. Remdesivir showed potent antiviral activity with an EC₅₀ value of 0.38 µM (Figure 1a), while DRV showed no effect (EC₅₀ >100 µM, Figure 1b). No cytotoxicity of remdesivir or DRV was observed on Caco-2 cells with CC₅₀ values >100 µM. The selectivity index (CC₅₀ / EC₅₀) for DRV could not be calculated (>100 µM / >100 µM for SARS-CoV-2) due to the lack of antiviral activity. In contrast, remdesivir had a selectivity index of >900 by visual CPE scoring and >260 by the MTT method, confirming a strong *in vitro* antiviral effect against SARS-CoV-2.

Modelling

In silico docking of DRV in the crystal structure of the SARS-CoV-2 main protease (3CL protease) identified five docking poses. The docking scores ranged from S= -8.6 to -8.2, showing that DRV can fit into the pocket, but these values are indicative of suboptimal binding to this protein. Visual inspection of each of these poses showed very few interactions of DRV with the active site of the protease, and the catalytic cysteine residue was not directly targeted, unlike the many strong interactions observed for DRV bound to the HIV protease (14).

Discussion

Current efforts to manage the COVID-19 pandemic have largely focused on improved hygiene, quarantine of infected individuals, social distancing to limit transmission and development of a vaccine (15). Despite the expedited efforts to develop a vaccine and collaborative efforts to screen compounds in discovery and development across the broader pharmaceutical industry for activity against COVID-19, patients are in immediate need of therapeutic interventions (12, 16).

Current data on the therapeutic effect of HIV protease inhibitors in patients with COVID-19 are far from comprehensive. This study demonstrated that DRV showed no *in vitro* antiviral activity against SARS-CoV-2 at clinically relevant concentrations. Furthermore, structural analyses using protease structures are consistent with these data. DRV binds to the active site of the HIV virus' dimeric aspartic protease (14). The crystal structure of this protease is well-elucidated and has been shown to have an extensive hydrogen-bonding network with DRV, allowing for the potent *in vitro* activity (EC_{50} values = 1.2 to 8.5 nM) of this protease inhibitor against HIV (6-7). By contrast, the SARS-CoV-2 main protease is a cysteine protease (Protein Data Bank-code 6LU7) and while several docking poses have been found for DRV in *in silico* models, unlike in HIV, these poses showed little interaction with the SARS-CoV-2 main protease active site. Several publications describe *in silico* docking experiments on the main coronavirus protease that specifically focus on or include DRV (17–21). Although these studies suggest DRV as a candidate for further investigation, such promising docking results could not be reproduced in our *in silico* docking studies. Such discrepancies can often result from *in silico* docking, which is primarily a useful approach for identifying subsets of molecules for *in vitro* activity testing. No *in vitro* antiviral activity of DRV against SARS-CoV-2 was found in the experiments reported here.

In this study, remdesivir demonstrated activity against SARS-CoV-2 with an EC₅₀ of 0.38 µM, which is in line with the earlier reported remdesivir EC₅₀ of 0.77 µM, indicating that the *in vitro* antiviral assay used is appropriate to assess antiviral activity against SARS-CoV-2 (12).

In conclusion, the lack of *in vitro* antiviral activity of DRV against SARS-CoV-2 does not support the use of DRV for treatment of COVID-19. Hence, the use of DRV (boosted with either ritonavir or cobicistat) should remain solely for treatment of patients with HIV infection.

Acknowledgments

Medical writing supporting for the development of this manuscript was provided by Patrick Hoggard of Zoetic Science, an Ashfield company, part of UDG Healthcare plc; this support was funded by Janssen Pharmaceuticals. We thank Lena Stegmann for technical support by antiviral assays.

Disclaimer

Sandra De Meyer, Christophe Buyck, Ellen Van Damme, Marnix Van Loock and Brian Woodfall are employees and may be stock owners of Johnson and Johnson. Sandra Ciesek received research funding from Janssen for this research.

Funding for this study was provided by Janssen Pharmaceutica.

The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at <https://www.janssen.com/clinical-trials/transparency>. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access (YODA) Project site at <http://yoda.yale.edu>.

Address for Correspondence

Sandra De Meyer, PhD,

Turnhoutseweg 30,

2340 Beerse

Belgium

sdmeyer@its.inj.com.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *New Engl J Med.* 2020;382:727–33.
2. Lu R, Zhao X, Juan L, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* 2020;395:565–74.
3. Hoehl S, Rabenau H, Berger A, Kortenbusch M, Cinatl J, Bojkova D, et al. Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China. *N Engl J Med.* 2020;328:1278-80. doi: 10.1056/NEJMc2001899.
4. Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, et al. Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. *Thorax.* 2004;59(3):252–6.
5. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. *New Engl J Med.* 2020 [Epub ahead of print]. DOI: 10.1056/NEJMoa2001282.
6. Prezcofix prescribing information.
https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/205395s001lbl.pdf
(Accessed Mar 27, 2020).
7. Rezolsta Summary of product characteristics.
https://www.ema.europa.eu/en/documents/product-information/rezolsta-epar-product-information_en.pdf (Accessed Mar 27, 2020)
8. Tashima K, Crofoot G, Tomaka FL, Kakuda TN, Brochot A, Van de Castele T, et al. Cobicistat-boosted darunavir in HIV-1-infected adults: week 48 results of a Phase IIIb, open-label single-arm trial. *AIDS Res Ther* 2014;11:39. doi: 10.1186/1742-6405-11-39

9. Orkin C, DeJesus E, Khanlou H, Stoehr A, Supparatpinyo K, Lathouwers E, et al. Final 192-week efficacy and safety of once-daily darunavir/ritonavir compared with lopinavir/ritonavir in HIV-1-infected treatment-naïve patients in the ARTEMIS trial. *HIV Med.* 2013;14(1):49–59. doi: 10.1111/j.1468-1293.2012
10. Cahn P, Fourie J, Grinsztejn B, Hodder S, Molina JM, Ruxrungtham K, et al. Week 48 analysis of once-daily vs. twice-daily darunavir/ritonavir in treatment-experienced HIV-1-infected patients. *AIDS.* 2011;25(7):929–39. doi: 10.1097/QAD.0b013e328345ee95
11. Navarro J, Curran A. Profile of once-daily darunavir/cobicistat fixed-dose combination for the treatment of HIV/AIDS. *HIV AIDS (Auckl).* 2016;8:175–82.
12. Bojkova D, Klann K, Koch B, Widera M, Krause D, Ciesek S, et al. SARS-CoV-2 infected host cell proteomics reveal potential therapy targets, [Under review] *Nature*, 2020 preprint available at <https://www.researchsquare.com/article/rs-17218/v1>.
13. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020;30(3):269–71. doi: 10.1038/s41422-020-0282-0
14. King NM, Prabu-Jeyabalan M, Nalivaika EA, Wigerinck P, de Béthune MP, Schiffer CA. Structural and thermodynamic basis for the binding of TMC114, a next-generation human immunodeficiency virus type 1 protease inhibitor. *J Virol.* 2004;78(21):12012–21.
15. Kruse RL. Therapeutic strategies in an outbreak scenario to treat the novel coronavirus originating in Wuhan, China. *F1000Res.* 2020 Jan 31;9:72. Doi. 10.12688/f1000research.22211.2.
16. Baden LR, Rubin EJ. Covid-19 – The search for effective therapy. *N Engl J Med.* 2020 Mar 18. doi: 10.1056/NEJMe2005477. [Epub ahead of print].
17. Chang Y-C, Tung Y-A, Lee K-H, Chen T-F, Hsiao Y-C, Chang H-C, et al. Potential therapeutic agents for COVID-19 based on the analysis of protease and RNA

polymerase docking. Preprints (www.preprints.org) 2020.

doi:10.20944/preprints202002.0242.v1.

18. Haitao Y, Zihe R. 2020. Available at Shanghai Institute of Materia Medica.

http://www.simm.ac.cn/xwzx/kydt/202001/t20200125_5494417.html.

19. Sang P, Tian S, Meng Z, Yang, L. 2020: Insight derived from molecular docking and molecular dynamics simulations into the binding interactions between HIV-1 protease inhibitors and SARS-CoV-2 3CLpro. ChemRxiv. Preprint.

https://chemrxiv.org/articles/Insight_Derived_from_Molecular_Docking_and_Molecular_Dynamics_Simulations_into_the_Binding_Interactions_Between_HIV-1_Protease_Inhibitors_and_SARS-CoV-2_3CLpro/11932995/1

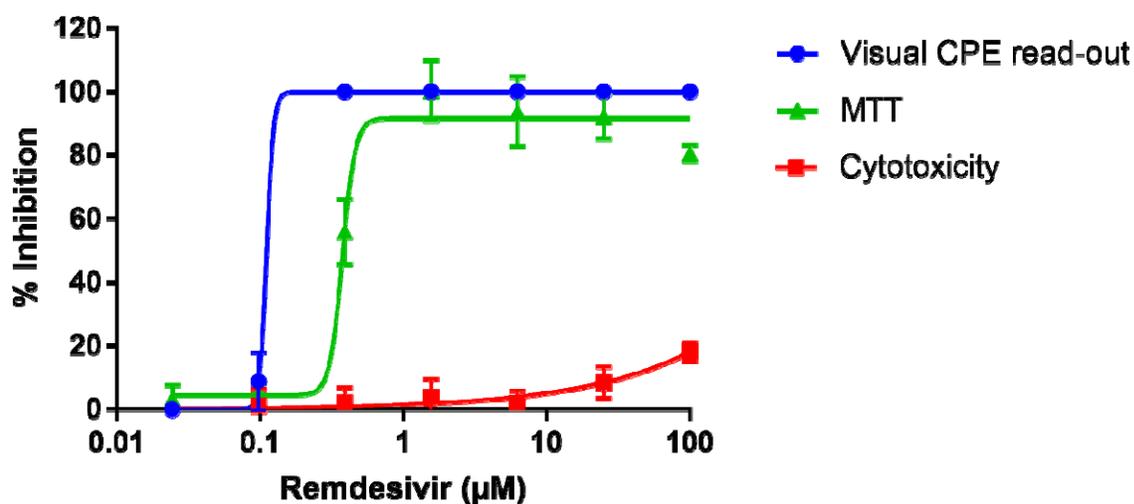
20. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharmaceutica Sinica B.2020. doi.org/10.1016/j.apsb.2020.02.008.

21. Farag A, Wang P, Ahmed M, Sadek H. Identification of FDA approved drugs targeting COVID-19 virus by structure-based drug repositioning. 2020.

https://chemrxiv.org/articles/Identification_of_FDA_Approved_Drugs_Targeting_COVID-19_Virus_by_Structure-Based_Drug_Repositioning/12003930/1.

1 **Figure 1.** Inhibition of SARS-CoV-2 in Caco-2 cells by cytopathogenic effect assay using a
2 visual read-out (visual CPE read-out) and MTT assay (MTT) following addition of a) remdesivir,
3 or b) darunavir (DRV). Cytotoxicity data (measured by MTT on uninfected cells) are also shown.
4 Mean percent inhibition for each readout across three independent experiments with triplicate
5 measurements are plotted (2 independent experiments for the MTT assay). The error bars
6 represent the standard deviation.

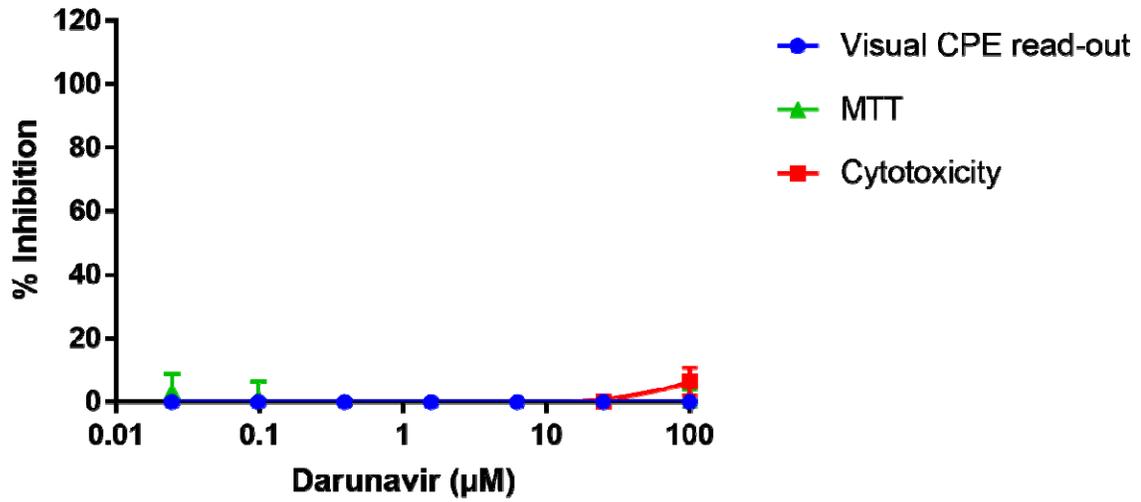
7 a)
8



9
10
11
12
13
14
15
16
17
18

Visual CPE-read out $EC_{50} = 0.11 \mu\text{M}$; MTT $EC_{50} = 0.38 \mu\text{M}$; $CC_{50} > 100 \mu\text{M}$;
Selectivity index = > 900 (Visual CPE read-out); > 260 (MTT)

19 b)
20
21



22
23
24
25
26 Visual CPE read-out $EC_{50} > 100 \mu\text{M}$; MTT $EC_{50} > 100 \mu\text{M}$; $CC_{50} > 100 \mu\text{M}$