SARS-CoV-2 pandemic : Time to revive the cyclophilin inhibitor alisporivir

Jean-Michel Pawlotsky[1,2]

[1]Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France;

[2]INSERM U955, Créteil, France

Correspondence: Prof. Jean-Michel Pawlotsky, MD, PhD, Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France.

Tel: +33-1-4981-2827; Fax: +33-1-4981-4831

E-mail : jean-michel.pawlotsky@aphp.fr

Summary

Alisporivir is a non-immunosuppressive analogue of cyclosporine A that potently inhibits cyclophilins. Cyclophilins play a major role in coronavirus lifecycles. Alisporivir inhibits several of them, including SARS-CoV-2, *in vitro*. Thus, alisporivir is a promising candidate for the treatment of COVID-19.

Abstract

December 2019 saw the emergence of a new epidemic of pneumonia of varying severity, called COVID-19, caused by a newly identified coronavirus, SARS-CoV-2. No therapeutic option is available to treat this infection that has already killed more than 235,000 people worldwide. This Viewpoint summarizes the strong scientific arguments supporting the use of alisporivir, a non-immunosuppressive analogue of cyclosporine A with potent cyclophilin inhibition properties that has reached Phase 3 clinical development, for the treatment of COVID-19. They include the strong cyclophilin dependency of the lifecycle of many coronaviruses, including SARS-CoV and MERS-CoV, and preclinical data showing strong antiviral and cytoprotective properties of alisporivir in various models of coronavirus infection, including SARS-CoV-2. Alisporivir should be tested without delay on both virological and clinical endpoints in patients with or at-risk of severe forms of SARS-CoV-2 infection.

December 2019 saw the emergence of a new epidemic of pneumonia of varying severity that started in the Chinese city of Wuhan [1]. The agent responsible for the epidemic was very rapidly identified [2]. It is a coronavirus, hitherto unknown member of the Orthocoronavirinae subfamily, genus beta-coronavirus, forming a separate clade within the sarbecovirus subgenus (lineage B) to which also belongs the virus responsible for the human "severe acute respiratory syndrome" (SARS-CoV) which killed nearly 800 people during an epidemic in 2003. The new virus has been called SARS-CoV-2 and the new disease Coronavirus Disease 2019 (COVID-19).

Despite initial measures aimed at preventing its spread, the disease is now pandemic, with active hotspots of infection in Western Europe and North America. The typical clinical presentation of COVID-19 is an acute respiratory illness with fever and respiratory symptoms, including cough and shortness of breath. The disease may aggravate and necessitate oxygen therapy and, especially in patients with risk factors (such as overweight, hypertension, diabetes or cardiac disease), transfer to an intensive care unit and mechanical ventilation. Milder forms may be limited to runny nose or sore throat, while an unknown proportion of patients remains asymptomatic. Non-respiratory, sometimes severe manifestations have also been described, including taste and/or olfactory disorders, as well as gastrointestinal, neurological, cardiovascular and/or ocular symptoms [1, 3]. At the time of writing this Viewpoint, more than 3.3 million people have been diagnosed with COVID-19 worldwide, although the exact figure is unknown, and over 235,000 of them died from complications from the disease [4].

Unfortunately, there is no known antiviral treatment for coronavirus infections. Several mechanisms have been identified as potential targets for direct-acting antivirals and host-targeted agents against SARS and the Middle-East Respiratory Syndrome coronavirus (MERS-CoV) [5]. However, these hypotheses could not be clinically evaluated, because these two epidemics spontaneously stopped their progression.

Empirical attempts have been made in the context of the COVID-19 epidemic to repurpose drugs that are approved or have reached late clinical developmental stages in other indications. The combination of the antiretroviral drug lopinavir with ritonavir failed to demonstrate antiviral efficacy in patients with severe COVID-19 disease [6]. Chloroquine, an anti-malarial drug, bears significant antiviral properties against SARS-CoV-2 *in vitro* [7]. Chloroquine has been tested in patients with COVID-19, alone or in combination with the anti-bacterial drug azithromycin, with thus far contradictory results, probably due to the heterogeneity of the populations studied (mild to severe, early to late disease) and methodological flaws [8]. Camostat mesylate was also recently suggested to act as a SARS-CoV, MERS-CoV and SARS-CoV-2 entry inhibitor *in vitro* [9], but no clinical data are available. The nucleotide analogue remdesivir showed antiviral efficacy against SARS-CoV and MERS-CoV viruses in both *in vitro* and animal models [10-13]. Remdesivir was recently reported to inhibit the SARS-CoV-2 lifecycle *in vitro* [7]. Modest but significant results from a randomized clinical trial were recently released: the administration of remdesivir was associated with a significantly shortened median time to recovery (11 *vs* 15 days) and a trend towards less frequent death (8.0% *vs* 11.6%, p=0.059) [14]. However, a yet unpublished Chinese study reported negative results with this compound. Overall, the antiviral treatment of COVID-19 remains to be found.

A very credible therapeutic option for SARS-CoV-2 is the use of cyclophilin inhibitors. Cyclophilins are host peptidyl-prolyl *cis-trans* isomerases. They catalyze the interconversion of the two energetically preferred conformers (*cis* and *trans*) of the planar peptide bond preceding an internal proline residue [15, 16]. Cyclophilins are structurally made up of a common domain surrounded by specific domains which define their subcellular compartmentalization and their functional specialization. Several cyclophilins play a central role in the lifecycle of viruses from different families, including HIV, hepatitis C virus (HCV), dengue virus, Japanese encephalitis virus, yellow fever virus, hepatitis B virus, cytomegalovirus, human papillomavirus 16, influenza A virus or vesicular stomatitis virus [17].

Cyclophilins also play a very important role in the lifecycle of many coronaviruses. It has indeed been shown that the lifecycles of human coronaviruses 229E (HCoV-229E) and NL-63 (HCoV-NL63), responsible for mild respiratory infections in humans, of feline infectious peritonitis coronavirus (FPIV), responsible for a fatal disease in cats, and of SARS-CoV were highly dependent on cyclophilin A (and possibly also cyclophilin B for FPIV) [18-22]. The N protein of SARS-CoV binds strongly to cyclophilin A and this binding could facilitate cell invasion [19, 23]. Interestingly, the SARS-CoV and MERS-CoV virions bring with them quantities of cyclophilin A sufficient for the achievement of their lifecycle, allowing them to compensate for a defect in cell production in their target cells [24]. In this context, it is not surprising that cyclosporin A (CsA), a potent cyclophilin inhibitor which also has immunosuppressive anti-calcineurin properties, inhibits the replication of various coronaviruses *in vitro*, including HCoV-229E, HCoV-NL63, FPIV, mouse hepatitis virus (MHV), avian infectious bronchitis virus (IBV), and SARS-CoV, the virus genetically closest to SARS-CoV-2 [22, 25, 26].

Because of its strong immunosuppressive properties, CsA cannot be considered as a therapeutic option for SARS-CoV-2 infections. There are, however, alternative molecules capable of strongly inhibiting the functional activity of cyclophilins without exerting any immunosuppressive effects. This is the case for the non-immunosuppressive analogue of CsA alisporivir (Debio 025). Alisporivir was developed by the Swiss company Debiopharm, initially for the treatment of chronic HCV infection, as the HCV lifecycle is strongly dependent on cyclophilin A. Alisporivir induced a decrease of HCV replication of the order of 3 to 4 logs in patients infected with all HCV genotypes, with a very high barrier to resistance since the drug target is a host protein [27, 28]. Administered for 14 days to 24 weeks as a monotherapy, alisporivir was well tolerated. Headache, nausea, fatigue and a few cases of reversible hyperbilirubinemia were the most common adverse events in patients treated with the highest dose of the drug [28-31]. Hyperbilirubinemia was not observed in previous studies with alisporivir at oral dosages up to 1,200 mg/day for 10 days [28]. We have shown that the combination of alisporivir and ribavirin is also very well tolerated for several weeks in HCV-infected patients [31].

Alisporivir has entered Phase 3 clinical evaluation for the treatment of chronic hepatitis C, in combination with pegylated interferon alpha and ribavirin, under license with Novartis. However, in April 2012, this Phase 3 program was interrupted due to the occurrence of 3 cases of acute pancreatitis, including one fatal case, occurring several weeks after the onset of treatment in a trial. Overall, there were 7 cases of acute pancreatitis among over 2,000 patients included in the alisporivir clinical development program, with equal frequency in patients receiving pegylated interferon alpha with or without alisporivir (0.35% *vs* 0.41%, respectively) [32]. Pancreatitis is a classic complication of interferon alpha and the direct responsibility of alisporivir in the reported cases has not been formally

established, especially since no case has occurred with alisporivir alone or with alisporivir and ribavirin in other studies [28, 31].

The current situation is that a non-immunosuppressive CsA analogue endowed with powerful cyclophilin inhibitory properties and having reached Phase 3 clinical development, which is well tolerated for several weeks of administration alone or in combination with ribavirin, exists. Strong arguments suggest that alisporivir will have antiviral efficacy against SARS-CoV-2 infections. First, the lifecycles of many coronaviruses, including SARS-CoV, are strongly dependent on cyclophilins. Secondly, experimental results, including findings from our laboratory, indicate that alisporivir inhibits the replication of HCoV-229E, HCoV-NL63 and MHV at low micromolar concentrations and without cytotoxic effect *in vitro* [18, 33, 34]. Thirdly, we observed that alisporivir fully abolishes the cytopathic effect of HCoV-229E in cell culture (unpublished data). A recent study showed that alisporivir blocks the lifecycle of SARS-CoV and MERS-CoV in different cellular models of infection [34]. However, treatment of a mouse model of SARS-CoV infection with alisporivir and ribavirin did not lead to a reduction in infection-related mortality or weight loss compared to untreated mice [34], emphasizing the importance of a careful design of clinical trials to maximize the clinical effect of the antiviral treatment.

The strongest argument in favor of the use of alisporivir in the treatment of COVID-19 is our demonstration of its antiviral effectiveness in VeroE6 cells infected with SARS-CoV-2. In this model, alisporivir inhibited SARS-CoV-2 lifecycle at an efficient concentration 50% (EC_{50}) of 0.46±0.04 µM and an EC_{90} of 3.10±1.40 µM, with a maximum viral load reduction of 2 logs [35]. For comparison, the EC_{50} of alisporivir against HCV was 0.04±0.03 µM in an HCV subgenomic replicon model in Huh7 cell line. Given the major differences between the models used for HCV and SARS-CoV-2, respectively, it is hard to speculate on the different

$EC_{50}s$ for these two viruses. Nevertheless, higher doses of alisporivir than those used for HCV will likely be required for the treatment of SARS-CoV-2 infection.

In a rat model, alisporivir appeared to accumulate in lung tissues (concentrations up to 37-fold higher than in plasma) (Debiopharm, data on file). Based on a human pharmacokinetic model and predicted accumulation in lung tissue, a dosing regimen of 600 mg twice per day or higher would achieve trough levels of unbound alisporivir above EC_{90} against SARS-CoV-2 at one week of treatment in the majority of patients. Alisporivir treatment should be started at rather early stages of the infection (i.e. prior to the need for mechanical ventilation) and administered for up to 14 days to pass the acute stage of clinical disease.

Alisporivir inhibits all cyclophilins, including cyclophilin D, an essential component of the mitochondrial permeability transition pore (mPTP). Cyclophilin D inhibition has been shown to be beneficial in heart, kidney and hepatic disorders in which abnormal mPTP opening plays a major pathogenic role [36, 37]. Whether similar protection of lung tissues will be achieved by alisporivir in order to improve the clinical course of COVID-19, in the presence or in the absence of a significant antiviral effect, must be assessed. Therefore, the endpoints of a clinical trial with alisporivir in patients infected with SARS-CoV-2 should not only be the effect on viral replication, but also the protective effect of treatment on lung disease and complications, as well as the clinical and functional improvement of patients, including proportion of patients developing respiratory failure, proportion of patients requiring invasive or noninvasive mechanical ventilation, and all-cause mortality. A Phase 2, proof-of-concept clinical trial assessing the safety and efficacy of alisporivir in the treatment of patients with COVID-19 is planned to start soon, if the current evolution of the epidemics does not prevent us from including the necessary number of patients.

Over 235,000 patients already died from SARS-CoV-2 infection on the day of writing this Viewpoint. If alisporivir proves to have an antiviral and/or clinical effect, we will, finally, have a first-line therapeutic solution to the current pandemic, as well as, possibly, a weapon against the future emergence of other coronaviruses. In the absence of an effect, we will have advanced scientific knowledge and, most importantly, we will have no regrets.

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