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Commentary

SARS-CoV-2 and HIV protease inhibitors: why lopinavir/ritonavir will not work for COVID-19 infection

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Since the beginning of the outbreak of severe acute respiratory syndrome (SARS) coronavirus (CoV) 2, lopinavir/ritonavir was selected for treatment. The recent publication of Cao *et al*. in the *New England Journal of Medicine* showed that lopinavir/ritonavir treatment did not accelerate clinical improvement compared with standard of care. This raised the question of whether in retrospect we could have known this. The aim of this paper is to gather all the available evidence and to comprehensively discuss this issue.

In December 2019, the first reports of severe acute respiratory syndrome (SARS) coronavirus (CoV) 2 infections were described. Since then, the virus rapidly expanded across the world, achieving pandemic status in March 2020.

From the beginning of the outbreak various antiviral treatment regimens have been identified for the treatment of SARS-CoV-2 infection, including antiviral agents (remdesivir, favipiravir), immunomodulating agents (interferons, tocilizumab) and a combination of both (ribavirin and chloroquine). These drugs were selected based on *in vitro* and *in vivo* research performed in the field of SARS-CoV and Middle East respiratory syndrome (MERS)-CoV, which caused coronavirus outbreaks in Asia (2003) and the Middle East region/South Korea (2012), respectively. Not without rational, HIV protease inhibitors (PIs) that prevent HIV viral replication by binding to its proteases, such as lopinavir, were initially suggested as antiviral agents against SARS-CoV-2.

The clinical use of lopinavir/ritonavir was quickly adapted for coronavirus disease 2019 (COVID-19) treatment when the virus arrived in the Netherlands in late February 2020. Lopinavir is the active PI which is pharmacokinetically enhanced by its co-formulated ritonavir [1]. In the scientific community other HIV PIs such as atazanavir and darunavir were also suggested as possible candidates for COVID-19 treatment. As all PIs inhibit HIV viral replication, why wouldn't they also inhibit SARS-CoV-2 replication? This hypothesis was revisited with the publication of Cao *et al*. [2] in the *New England Journal of Medicine* of 18 March 2020. This randomized controlled open-label trial showed that lopinavir/ritonavir treatment does not significantly accelerate clinical improvement (16 versus 16 days), reduce mortality (19.2 versus 25%) or diminish throat viral RNA detectability (day 28, 60.3 versus 58.6%) in patients with serious COVID-19 compared with standard of care [2]. The authors acknowledged that the study suffered from limitations, but results were a signal that lopinavir/ritonavir was not beneficial for COVID-19 treatment [2]. Lopinavir/ritonavir is still used for COVID-19 treatment but, in the Netherlands, the publication of Cao *et al*. [2] caused lopinavir/ritonavir to be removed from the national treatment guidelines.

In retrospect, the question has been raised, why does lopinavir/ritonavir not work for the treatment of COVID-19 disease and could we have known this upfront?

We extensively searched PubMed, web-based search engines (Google, Google Scholar) and preprint servers (medRxiv; bioRxiv) for publications on the use of HIV PIs for SARS-CoV, MERS-CoV and SARS-CoV-2 treatment. We searched for all relevant publications up to 8 May 2020 by snowballing.

Data for SARS-CoV, MERS-CoV and SARS-CoV-2 are summarized in *Overview of published in silico, in vitro, and in vivo data of protease inhibitors for the treatment of SARS-CoV, MERS-CoV, and SARS-CoV-2*

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in Additional file 1, Table 1, and could be categorized in *in silico* (*n*=2 versus *n*=0 versus *n*=1), *in vitro* (*n*=4 versus *n*=3 versus *n*=3) and *in vivo* (*n*=1 versus *n*=1 versus *n*=8) evidence. *In vitro* evidence of lopinavir's activity against SARS-CoV is contradictory. Yamamoto *et al.* [3] found that lopinavir did not affect SARS-CoV replication, as measured by a reduction in cell viability. Other researchers reported *in vitro* half maximum effective concentrations (EC_{50}) for antiviral activity against SARS-CoV varying from 4.0 to 10.74 mg/l [4–6]. Chu *et al.* [4] found that the EC_{50} could be further reduced from 4 mg/l to 1 mg/l when lopinavir was combined with ribavirin, suggesting a synergistic effect. EC_{50} values for antiviral activity of lopinavir of 5.03–7.29 mg/l against MERS-CoV have been reported [5,7].

To predict *in vivo* efficacy of lopinavir against SARS-CoV-2, reported *in vitro* EC_{50} values found against SARS-CoV and MERS [4–7] were compared with clinical total and (predicted) unbound maximal and minimal plasma concentrations (C_{max} and C_{min}) reported in HIV-infected patients. C_{max} and C_{min} after intake of 400/100 mg lopinavir/ritonavir twice daily in HIV patients were 9.8 and 5.5 mg/l with a protein binding of 98–99% [1]. In this same dose, lopinavir has an intracellular/plasma concentration ratio of 1.18 in peripheral blood mononuclear cells (PBMCs), not supporting a large difference between tissue and plasma concentrations [8].

It is important that HIV patients are, in general, relatively healthy individuals who may not be comparable with critically ill COVID-19 patients admitted to the intensive care units. These pathophysiological changes may also significantly influence pharmacokinetics.

Data initially available for the use of lopinavir against SARS-CoV-2 were mainly based on *in vitro* activity against SARS-CoV and MERS-CoV and were used to predict *in vivo* efficacy retrospectively. At the moment of writing this paper, both *in vitro* SARS-CoV-2 data became available for lopinavir (EC_{50} 3.6 mg/l) [9] and pharmacokinetic data of eight COVID-19 patients treated with 400/100 mg lopinavir twice daily. The median C_{min} was 13.6 mg/l with a range from 6.2 to 23.4 mg/l, which is an approximately twofold increase in C_{min} compared with HIV patients [10] (*Overview of in vivo pharmacokinetic parameters in HIV-infected patients compared to in vitro antiviral activity against SARS-CoV-2* in Additional file 1, Table 2).

Translation of such an *in vitro* parameter to clinical practice should be made cautiously considering its limited protein binding compared with plasma pharmacokinetic parameters (C_{max} and C_{min}). Most clinical studies report total concentrations (bound + unbound), while *in vitro* only a relatively small amount of protein is present, that is, cell culture medium is supplemented with diluted (5–10%) animal serum. The unbound fraction of a drug is pharmacologically active, and therefore the comparison between EC_{50} values and unbound plasma concentrations is probably more appropriate. These values reported for lopinavir against the coronaviruses are in the same concentration range as the total C_{max}. This most likely means that *in vivo* free lopinavir plasma concentrations are too low for effective reduction of viral replication. In line with this, the PI darunavir had an EC_{50} of approximately 55–165 mg/l reported, largely exceeding darunavir intracellular and plasma concentrations, meaning that darunavir is not a suitable candidate for COVID-19 therapy.

Comparisons between EC_{50} and C_{max} were made to make assumptions about the pharmacokinetic–pharmacodynamic (PK–PD) relationship between lopinavir concentrations and SARS-CoV-2 viral load reductions. Important for the interpretation of the PK–PD relationship is that the EC_{50} represents the concentration where *in vitro* 50% reduction of viral replication is achieved. Preferably the C_{max} is higher than the EC_{50} and actually an EC_{90} would be preferred. However, EC_{90} values are more difficult to assess and are often not reported.

In general, when pharmaceutical companies develop antiviral drugs they aim for plasma concentrations greatly exceeding the IC_{90} and the FDA states that a 'high' inhibitory quotient (C_{min}/EC_{50}) indicates effective compound concentrations with minimal risk of resistance [11]. In addition, it is stated for HIV antiretroviral drug development that dosages chosen for Phase II trials should exceed, by several-fold, the protein binding adjusted EC_{50} [12]. Exceeding IC_{90} is needed to accomplish a clinically relevant viral load reduction (meaning undetectable). When comparing with HIV, the C_{min} (5.5 mg/l) of lopinavir exceeded >75-fold the IC_{50} (0.007 mg/l) of lopinavir for wild-type HIV resulting in undetectable HIV viral load. From a previous study in HIV-infected patients it is known that lopinavir/ritonavir activity is highest when IC_{50} values of HIV isolates were <10-fold increased compared with the wild type; with increasing isolate IC_{50} values the susceptibly to lopinavir decreased [13]. Based on these data it seemed unrealistic to expect any antiviral efficacy of lopinavir/ritonavir against SARS-CoV-2 as the EC_{50} of lopinavir against SARS-CoV and MERS-CoV were approximately 4 to 10 mg/l and 5 to 7 mg/l, respectively, which is in the same concentration range as observed lopinavir C_{min} concentrations. In addition, we also know from HIV therapy that the C_{min} of lopinavir is an important predictor of efficacy and that the inhibitory quotient should be at least >15 [14]. This quotient takes, among others, resistance and drug distribution into cells into account. In this context, a C_{min}/EC_{50} ratio for lopinavir and SARS-CoV-2 of 3.8 is most likely insufficient. Such an extrapolation from the field of HIV seems warranted considering the comparable mechanism; inhibiting the viral protease instead of 'killing' an organism.

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Of note, both plasma and intracellular (PBMC) concentrations are surrogate markers for tissue concentrations. Currently, it is unknown if and how lopinavir distributes to the site of infection, this it, the lung in critically ill COVID-19 patients.

An important clinical consideration is the interpretation of the findings by Cao *et al*. [2], in that patients were treated with monotherapy lopinavir/ritonavir, while other published studies in COVID-19 all used combination therapy (*Overview of published in silico, in vitro, and in vivo data of protease inhibitors for the treatment of SARS-CoV, MERS-CoV, and SARS-CoV-2* in Additional file 1, Table 1). This could be an interesting approach to evaluate SARS-CoV-2 treatment, as combining lopinavir/ritonavir with other agents might cause a synergistic effect, which was also suggested in an *in vitro* study combining lopinavir with ribavirin [4].

Overall it seems that at least lopinavir/ritonavir as monotherapy would not be effective for COVID-19 treatment. Nonetheless, results of more trials remain highly anticipated, and >30 studies with lopinavir/ritonavir (and other antivirals) in COVID-19 are registered on ClinicalTrials.gov.

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Additional file

Additional file 1: Supplementary tables can be found at https://www.intmedpress.com/uploads/documents/4738_Smolders_Addfile1.pdf

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