Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Does lopinavir really inhibit SARS-CoV-2?

HIV protease inhibitors are being considered as potential treatment of COVID-19, the disease caused by Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2). In particular, the use of lopinavir/ritonavir has been supported by *in vitro data*, animal studies and clinical data in patients with other coronavirus infections, specifically SARS and Middle East Respiratory Syndrome (reviewed in [1]). However, early experiences with the use of lopinavir/ritonavir in COVID-19 patients provided conflicting results. Indeed, Ye et al. showed that, compared with treatment of pneumonia-associated adjuvant drugs alone, the association with lopinavir/ritonavir had more favorable effects in lowering the body temperature [2]. Conversely, Cao and co-workers, in a prospective, randomized, open-label trial, reported no added benefit of lopinavir-ritonavir in hospitalized adult patients with severe Covid-19 treated with standard of care [3]. We believe that such findings could have been predicted *a priori* just looking at available *in vitro/in vivo* data.

Lopinavir was identified as a potential treatment of SARS in 2003, with a half maximal inhibitory concentration (IC_{50}) of 50 micromolar (μM) [4]. More recently the *in vitro* antiviral effect of lopinavir was tested also against SARS-CoV-2, resulting in an IC_{50} of 26 μM [5]. Considering that COVID-19 patients are treated with the same lopinavir/ritonavir doses used for the treatment of HIV (400/100 mg twice daily), we compared the IC_{50} values found for SARS-CoV and SARS-CoV-2 with those found for the inhibition of HIV. The IC_{50} of lopinavir/ritonavir for HIV is 0.006 μM, a concentration that is 4000-to-8000 folds lower than those able to inhibit SARS-CoV and SARS-CoV-2 (Table 1) [4–6].

So, the key question is: what is the minimum concentration of lopinavir able to effectively inhibit SARS-CoV replication *in vivo*? To address this issue, three factors need to be considered. Firstly, the IC_{50} is not the ideal pharmacodynamic parameter because at this concentration 50 % of the virus still replicates; therefore, the IC_{90} should preferably be considered. Accordingly, the lopinavir IC_{90} values were estimated from IC_{50} considering a Hill slope factor of 1 (using https://www.graphpad.com/quickcalcs/Ecanything1.cfm). Secondly, lopinavir, circulates in the body largely bound plasma proteins (> 95 %) and its inhibitory activity on virus replication relies on the 5% or less free drug fraction. Therefore, the IC values need to be adjusted for serum protein content. Thirdly, drug concentrations at the site of infection should be properly considered. To address these two latter issues, we estimated the lopinavir protein-adjusted IC_{90} in the lung and the central nervous systems, considering the epithelial lining fluid (ELF)-to-plasma and cerebrospinal fluid (CSF)-to-plasma ratios of 1.7 and 0.004 [7,8]. We also assumed that lopinavir free fractions in ELF and in CSF were 35 % and 100 %, respectively, based on the fact that the concentrations of albumin in ELF and CSF are approximately 7-fold and 200-fold lower than plasma [9,10].

As shown in Table 1, the *in vitro* protein-adjusted IC_{90} values for HIV, SARS-CoV and SARS-CoV-2 were 1.00, 9000 and 4680 μM in plasma, 0.14, 756 and 393 in ELF, and 12.5, 112,500 and 58,500 in CSF. Lopinavir plasma concentrations measured *in vivo* in HIV-infected and in COVID-19 patients ranged from 1 to 20 μM and 10–40 μM, respectively [11,12].

Considered together, these data clearly indicate that the current dose of lopinavir provides effective concentrations exceeding the protein adjusted IC_{90} only for HIV. Conversely, the protein-adjusted IC_{90} values of lopinavir required to inhibit SARS-CoV-2 replication in plasma, ELF and CSF are, respectively, 200-fold, 20-fold and 2000-fold higher than the concentrations measured in vivo in COVID-19 patients. It is, therefore, not surprising that lopinavir was ineffective for the treatment of SARS-CoV-2, as Cao documented. The doses required to provide optimal inhibition are obviously impracticable due to un-

Table 1
In vitro pharmacodynamics and *in vivo* pharmacokinetics of lopinavir as treatment for HIV, SARS-CoV and SARS-CoV-2 infections. Lopinavir inhibitor concentration $(IC)_{90}$ was estimated from IC_{50} considering a Hill slope factor of 1; protein-adjusted ICs in plasma, in the epithelial lining fluid and in the cerebrospinal fluid were estimated considering lopinavir free fraction of 5%, 35 % and 100 % respectively.

PK/PD parameters	HIV (wild-type)	SARS-CoV	SARS-CoV-2
In vitro, lopinavir IC_{50}, μM	0.006	50	26
In vitro, lopinavir IC_{90}, μM	0.05	450	234
In vitro, lopinavir PA-IC_{90}, (plasma) μM	1.00	9000	4680
In vitro, lopinavir PA-IC_{90}, (ELF) μM	0.14	756	393
In vitro, lopinavir PA-IC_{90}, (CSF) μM	12.5	112,500	58,500
In vivo, lopinavir trough, μM*	1–20	Not available	10–50

IC: inhibitory concentration; PA: protein-adjusted; PK/PD: pharmacokinetic/pharmacodynamics; ELF: epithelial lining fluid; CSF: cerebrospinal fluid.
* Concentrations measured in patients given lopinavir/ritonavir at 400/100 mg twice daily.

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acceptable risk of toxicity. These limitations need to be carefully considered before to embarking in the planning of large scale, randomized controlled clinical trials including lopinavir-ritonavir as one of the key treatment arms for COVID-19 [13].

Declaration of competing interest

There are no conflicts to declare.

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