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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
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\textsubscript{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\textsubscript{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\textsubscript{50} values found for SARS-CoV and SARS-CoV-2 with those found for the inhibition of HIV. The IC\textsubscript{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-2 replication in vivo? To address this issue, three factors need to be considered. Firstly, the IC\textsubscript{50} is not the ideal pharmacodynamic parameter because at this concentration 50 % of the virus still replicates; therefore, the IC\textsubscript{90} should preferably be considered. Accordingly, the lopinavir IC\textsubscript{90} values were estimated from IC\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{90} only for HIV. Conversely, the protein-adjusted IC\textsubscript{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-
acceptable risk of toxicity. These limitations need to be carefully considered before 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.

References

[1] N. Ford, M. Vitoria, A. Rangaraj, S.L. Norris, A. Calmy, M. Doherty. Systematic review of the efficacy and safety of antiretroviral drugs against SARS, MERS or COVID-19: initial assessment, J. Int. AIDS Soc. 23 (4) (2020) e25489, https://doi.org/10.1002/jia2.25489.
[2] X.T. Ye, Y.L. Luo, S.C. Xia, Q.F. Sun, J.G. Ding, Y. Zhou, W. Chen, X.F. Wang, W.W. Zhang, W.J. Du, Z.W. Ruan, L. Hong. Clinical efficacy of lopinavir/ritonavir in the treatment of Coronavirus disease 2019, Eur. Rev. Med. Pharmacol. Sci. 24 (2020) 3390–3396.
[3] B. Cao, Y. Wang, D. Wen, W. Liu, J. Wang, G. Fan, L. Ruan, B. Song, Y. Cai, M. Wei, X. Li, J. Xia, N. Chen, J. Xiang, T. Yu, T. Bai, X. Xie, L. Zhang, C. Li, Y. Yuan, H. Chen, H. Li, H. Huang, S. Tu, F. Gong, Y. Liu, Y. Wei, C. Dong, F. Zhou, X. Gu, J. Xu, Z. Liu, Y. Zhang, H. Li, L. Shang, K. Wang, K. Li, X. Zhou, X. Dong, Z. Qiu, S. Lu, X. Hu, S. Ruan, S. Luo, J. Wu, L. Peng, F. Cheng, L. Pan, J. Zou, C. Jia, J. Wang, X. Liu, S. Wang, X. Wu, Q. Ge, J. He, H. Zhan, F. Qiu, L. Guo, C. Huang, T. Jaki, P.G. Hayden, P.W. Horby, D. Zhang, C. Wang. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19, N. Engl. J. Med. (2020), https://doi.org/10.1056/NEJMoa2001282 [Epub ahead of print].
[4] C.Y. Wu, J.T. Jan, S.H. Ma, C.J. Kuo, H.F. Juan, Y.S. Cheng, H.H. Hsu, H.C. Huang, D. Wu, A. Brik, F.S. Liang, R.S. Liu, J.M. Fang, S.T. Chen, P.H. Liang, C.H. Wong. Small molecules targeting severe acute respiratory syndrome human coronavirus, Proc Natl Acad Sci U S A. 101 (2004) 10012–10017.
[5] K.T. Choy, A. Yin-Lam Wong, P. Kaewpreedee, S.F. Sia, D. Chen, K.P.Y. Hui, D.K.W. Chu, M.C.W. Chan, P.P. Cheung, X. Huang, M. Peiris, H.L. Yen. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro, Antiviral Res. (2020), https://doi.org/10.1016/j.antiviral.2020.104786 [Epub ahead of print].
[6] Kaletra drug monograph. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021251s052_021906s046lbl.pdf (last access, April 1, 2020).
[7] C. Atzori, P. Villani, M. Regazzi, M. Maruzzi, A. Cargnel. Detection of intrapulmonary concentration of lopinavir in an HIV-infected patient, AIDS. 17 (2003) 1710–1711.
[8] J.M. Tiraboschi, H. Knobel, A. Imar, J. Villar, E. Ferrer, M. Saumoy, A. González, N. Rozas, A. Vila, J. Niubó, J. Curto, D. Podzamczer. Cerebrospinal fluid and plasma lopinavir concentrations and viral response in virologically suppressed patients switching to lopinavir/ritonavir monotherapy once daily, Antivir. Ther. (Lond.) 21 (2016) 359–363.
[9] C.M. Roberts, D. Cairns, B.H. Bryant, W.M. Burke, M. Yeates, H. Blake, R. Penny, L. Shelley, J.J. Saunders, S.N. Brit, Changes in epithelial lining fluid albumin associated with smoking and interstitial lung disease, Eur. Respir. J. 6 (1993) 110–115.
[10] M. Ishikawa, S. Yamauchi, T. Suzuki, M. Uchida, Y. Iwadeate, I. Ishii. Correlation between vancomycin penetration into cerebrospinal fluid and protein concentration in cerebrospinal fluid/serum albumin ratio. J. Infect. Chemother. 25 (2019) 124–128.
[11] D. Cattaneo, S. Baldelli, S. Castoldi, N. Charbe, V. Cozzi, S. Facile, E. Clementi. Is it time to revise antiretrovirals dosing? A pharmacokinetic viewpoint, AIDS. 28 (2014) 2477–2480.
[12] S. Baldelli, M. Corbellino, E. Clementi, D. Cattaneo, C. Gervasoni. Lopinavir/ritonavir in Covid-19 patients: maybe yes, but at what dose? J. Antimicrob. Chemother. (2020) in press.
[13] Kupferschmidt K., Cohen, J. WHO launches global megatриal of the four most promising coronavirus treatments. Science Mag. Available at https://www.sciencemag.org/news/2020/03/who-launches-global-megatrial-four-most-promising-coronavirus-treatments.

Dario Cattaneo1
1 Unit of Clinical Pharmacology, Fatebenefratelli Sacco University Hospital, Milan, Italy

Dario Cattaneo2, Cristina Gervasoni2,∗
2 Gestione Ambulatoriale Politerapie (GAP) Outpatient Clinic, ASST Fatebenefratelli Sacco University Hospital, Milan, Italy
E-mail address: cristina.gervasoni@unimi.it (C. Gervasoni).

Mario Corbellino1, Massimo Galli1, Agostino Riva1, Cristina Gervasoni1
1 3rd Division of Infectious Diseases, ASST Fatebenefratelli Sacco University Hospital, Milan, Italy

Emilio Clementi4
4 Unit of Clinical Pharmacology Fatebenefratelli Sacco University Hospital, Department of Biomedical and Clinical Sciences L. Sacco, Università di Milano, Milan, Italy

Emilio Clementi5
5 IRCCS E. Nedea Scientific Institute, Bosistio Parini, Italy

∗Correspondence to: 3rd Division of Infectious Diseases, ASST Fatebenefratelli Sacco University Hospital, Via GB Grassi 74, 20157 Milano, Italy.

2