Nelfinavir is active against SARS-CoV-2 in Vero E6 cells

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There are 462,684 confirmed COVID-19 (coronavirus disease 2019) cases and 20,834 deaths worldwide caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) as of March 26 2020.[1] Although several old drugs approved by FDA are currently undergoing clinical studies, none of them has been tested to be specifically effective by double-blind randomized controlled trial, while remdesivir is still on clinic trial. Therefore, more drug candidates are required for systematic evaluation as potential treatments of COVID-19.

Utilizing an integrative computational drug discovery approach, we predicted that nelfinavir is a potential inhibitor of SARS-CoV-2 main protease.[2] Further docking nelfinavir to 30 potential target proteins of COVID-19,[3] we found that nelfinavir is most probably a multi-target agent. Therefore, its antiviral activity was performed and repeated three times in duplicates in Vero E6 cells. The SARS-CoV-2 virus was isolated from a clinical isolate of SARS-CoV-2 infected patient. With remdesivir as positive control, the half-maximal effective concentration (EC_{50}) of nelfinavir mesylate against the SARS-CoV-2 was determined to be 2.89±0.65 μM while that of remdesivir was 1.00±0.34 μM, both drugs showed similar dose-response curves. For testing its cytotoxicity, the half-cytotoxic concentration of nelfinavir mesylate was measured with Vero E6 cells by CCK-8 assays. In agreement with the good safety profile observed in

clinic, the CC_{50} value of nelfinavir was determined to be 51.55±13.52 μM. Accordingly, the selectivity index (SI) was estimated to be 18.

Nelfinavir is a potent and orally bioavailable HIV-1 protease inhibitor, which was approved by FDA in 1997 for the treatment of HIV infection in children 2 years of age and older and adults. Nelfinavir in a clinic trial showed remarkably high peak and trough concentrations with values of 13.3 μM and ~5.5 μM, respectively, at a dose of 1875 mg BID,[4] which is higher than its *in vitro* EC_{50} value (2.89±0.65 μM) against SARS-CoV-2.

Nelfinavir was detectable in bronchoalveolar lavage (BAL) fluid in 100% patients treated at 4 weeks while lopinavir-ritonavir were detectable in BAL in only 16.7% patients.[5] The concentration of nelfinavir in the lung epithelial lining fluid was found to be similar to the concentration found in plasma,[5] showing the high penetration capability of nelfinavir into the alveolar compartment.

In peripheral blood mononuclear cells (PBMCs), the mean intracellular $AUC_{0–12}$ (area under the concentration-time curve from time zero to 12 hours), C_{min} (minimum concentration), C_0 (concentration at time zero) and C_{max} (maximum concentration) values of nelfinavir were found to be about 9-, 5-, 6- and 15-fold higher than that of plasma, respectively.[6] In another study, the cellular accumulation of nelfinavir in PBMCs was 5.30-fold compared to that in plasma.[7] Distribution study with rat revealed that the concentration of nelfinavir in lungs of rat is around 3 times as high as that in plasma.[8]

The cytokine storm has been associated with the disease severity of COVID-19, which could result in acute respiratory distress syndrome.[9,10] Nelfinavir was reported to effectively inhibit inflammatory cytokines at 2.5 μM *in vitro*, and to reduce inflammatory cytokine in a cohort of 31 pediatric HIV-1 patients for over 2 years of therapy.[11]

Based on its high potency against SARS-CoV-2 in Vero E6 cells, its higher exposure in lung than in plasma and its good safe profile, nelfinavir deserves further exploration as potential treatment of COVID-19.

References

1. WHO. Coronavirus disease (COVID-2019) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ (accessed Mar 27, 2020).

2. Xu, Z.; Peng, C.; Shi, Y.; Zhu, Z.; Mu, K.; Wang, X.; Zhu, W., Nelfinavir was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. *bioRxiv* **2020**, 2020.01.27.921627. DOI: 10.1101/2020.01.27.921627

3. Shi, Y.; Zhang, X.; Mu, K.; Peng, C.; Zhu, Z.; Wang, X.; Yang, Y.; Xu, Z.; Zhu, W., D3Targets-2019-nCoV: A Web Server to Identify Potential Targets for Antivirals Against 2019-nCoV. *ChemRxiv*. **2020**, DOI: 10.26434/chemrxiv.11831163.v1.

4. Driessen, C.; Kraus, M.; Joerger, M.; Rosing, H.; Bader, J.; Hitz, F.; Berset, C.; Xyrafas, A.; Hawle, H.; Berthod, G.; Overkleeft, H. S.; Sessa, C.; Huitema, A.; Pabst, T.; von Moos, R.; Hess, D.; Mey, U. J., Treatment with the HIV protease inhibitor nelfinavir triggers the unfolded protein response and may overcome proteasome inhibitor resistance of multiple myeloma in combination with bortezomib: a phase I trial (SAKK 65/08). *Haematologica* **2016,** *101* (3), 346-55.

5. Twigg, H. L.; Schnizlein-Bick, C. T.; Weiden, M.; Valentine, F.; Wheat, J.; Day, R. B.; Rominger, H.; Zheng, L.; Collman, R. G.; Coombs, R. W.; Bucy, R. P.; Rezk, N. L.; Kashuba, A. D., Measurement of antiretroviral drugs in the lungs of HIV-infected patients. *HIV Ther.* **2010,** *4* (2), 247-251.

6. Hennessy, M.; Clarke, S.; Spiers, J. P.; Kelleher, D.; Mulcahy, F.; Hoggard, P.; Back, D.; Barry, M., Intracellular accumulation of nelfinavir and its relationship to P-glycoprotein expression and function in HIV-infected patients. *Antivir. Ther.* **2004,** *9* (1), 115-22.

7. Ford, J.; Cornforth, D.; Hoggard, P. G.; Cuthbertson, Z.; Meaden, E. R.; Williams, I.; Johnson, M.; Daniels, E.; Hsyu, P.; Back, D. J.; Khoo, S. H., Intracellular and plasma pharmacokinetics of nelfinavir and M8 in HIV-infected patients: relationship with P-glycoprotein expression. *Antivir. Ther.* **2004,** *9* (1), 77-84.

8. Shetty, B. V.; Kosa, M. B.; Khalil, D. A.; Webber, S., Preclinical pharmacokinetics and distribution to tissue of AG1343, an inhibitor of human immunodeficiency virus type 1 protease. *Antimicrob. Agents Chemother.* **1996,** *40* (1), 110-4.

9. Xu, Z.; Shi, L.; Wang, Y.; Zhang, J.; Huang, L.; Zhang, C.; Liu, S.; Zhao, P.; Liu, H.; Zhu, L.; Tai, Y.; Bai, C.; Gao, T.; Song, J.; Xia, P.; Dong, J.; Zhao, J.; Wang, F. S., Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* **2020,** DOI: 10.1016/S2213-2600(20)30076-X.

10. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; Cheng, Z.; Yu, T.; Xia, J.; Wei, Y.; Wu, W.; Xie, X.; Yin, W.; Li, H.; Liu, M.; Xiao, Y.; Gao, H.; Guo, L.; Xie, J.; Wang, G.; Jiang, R.; Gao, Z.; Jin, Q.; Wang, J.; Cao, B., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **2020,** *395* (10223), 497-506.

11. Wallet, M. A.; Reist, C. M.; Williams, J. C.; Appelberg, S.; Guiulfo, G. L.; Gardner, B.; Sleasman, J. W.; Goodenow, M. M., The HIV-1 protease inhibitor nelfinavir activates PP2 and inhibits MAPK signaling in macrophages: a pathway to reduce

inflammation. *J. Leukocyte Biol.* **2012,** *92* (4), 795-805.
12. https://clinicaltrials.gov/ct2/show/NCT02066311.