Preprinted at https://doi.org/10.26434/chemrxiv.12039888.v1

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

Zhijian Xu[#,1], Hangping Yao[#,2,3], Jingshan Shen[1], Nanping Wu[2,3], Yechun Xu[1], Xiangyun Lu[2,3], Weiliang Zhu[*,1], Lan-Juan Li[*,2,3]

[1]CAS Key Laboratory of Receptor Research; Drug Discovery and Design Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China

[2]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases and [3]National Clinical Research Center for Infectious Diseases, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, China

[#]These authors contributed equally to this work. *Corresponding authors.

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.

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