HIV protease inhibitors saquinavir and nelfinavir are potent inhibitors of cathepsin L activity: A potential treatment for COVID-19 patients

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Short Report

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Abstract

The 2019 coronavirus disease pandemic (COVID-19) has mobilized efforts worldwide, and several ongoing clinical trials aimed at developing a drug-based treatment for its control. Cathepsin L is an endosomal cysteine protease that mediates the cleavage of the S1 subunit of the coronavirus surface spike glycoprotein. This cleavage is necessary for coronavirus entry into human host cells and viruses/host cell endosome membrane fusion. Therefore, cathepsin L is a potential target for the treatment of COVID-19 patients. In this report, we describe a previously unknown inhibitory effect of two FDA-approved drugs, saquinavir and nelfinavir, on human cathepsin L activity. Whether the pivotal role for cathepsin L in Sars-Cov-2 infection described in vitro can be translated to humans, our results support immediate clinical trials of saquinavir or nelfinavir as a potential treatment for COVID-19 patients.

Introduction

The 2019 coronavirus disease pandemic (COVID-19) has mobilized efforts worldwide, and several ongoing clinical trials aimed at developing a vaccine or drug-based treatment for its control. In a seminal paper, Hoffmann and colleagues demonstrated that SARS-CoV-2 employs the host cell surface receptor angiotensin-converting enzyme 2 (ACE2) for cell entry, and surface transmembrane serine protease 2 (TMPRSS2) for viral spike (S) glycoprotein priming. These authors also reported that clinically proven serine protease inhibitor camostat, which is active against TMPRSS2, partially blocked SARS-2-S-driven entry into cells. Although not highlighted in their report, full inhibition was obtained when the cathepsin B/L inhibitor E64 was used in combination, suggesting synergistic effects of TMPRSS2 and cathepsin B/L inhibitors. Similarly, Ou and colleagues show that cathepsin L inhibitor SID26681509 dramatically decreased SARS-CoV-2-S pseudovirions entry into 293/hACE2 cells, suggesting that cathepsin L is essential for priming of SARS-CoV-2 S protein in the lysosome. Indeed, this potential association has been recently proposed by Liu T et al. We have previously shown that the first generation HIV protease inhibitor, saquinavir, blocks the activity of mouse cathepsin L (cathepsin V in humans) in macrophages. Herein, we evaluated a possible inhibitory effect of several FDA-approved HIV protease inhibitors on the activity of cathepsin L.

Materials And Methods

Assessment of mouse cathepsin L activity

In brief, human cathepsin L was reconstituted directly in assay buffer (50 mM MES, 5 mM DTT, 1 mM EDTA) and kept on ice for 20 min. The reconstituted enzyme was mixed with vehicle (DMSO 0.1%) or increasing concentrations of inhibitor (0.1; 0.3; 1; 3; 10; 30 µM) followed by additional incubation on ice for 20 min. Afterward, the fluorogenic peptide substrate Z-LR-AMC was added, and activity assessed for 10 min using standard 96 wells reader fluorometer device. The final amount of cathepsin L was 8 ng per well and 10µM for the fluorogenic substrate. We calculate the IC50 using GraphPad Prism 8, U.S.A.
Statistical analysis

The results are expressed as means ± S.E.M. The comparisons between groups were assessed by two-way analysis of variance using Bonferroni correction or one-way analysis of variance followed by Dunnett's multiple comparison test. A probability value <0.05 was considered significant.

Results And Discussion

In this report, we describe an inhibitory effect of two FDA-approved drugs, saquinavir and nelfinavir, on human cathepsin L activity. Using recombinant human cathepsin L, standardized fluorescent substrate, and fluorescence spectroscopy techniques, we tested saquinavir, indinavir, ritonavir, darunavir, lopinavir, atazanavir, and nelfinavir. Additionally, a newly designed compound based on the saquinavir molecule named M374.1\(^5\) was tested, with the established cathepsin-L inhibitor, SID26681509, used as a positive control. While ritonavir, darunavir, lopinavir, indinavir, and atazanavir showed no or weak inhibitory effect on cathepsin L (less than 30%), saquinavir, nelfinavir, and M374.1 significantly inhibited cathepsin L (IC\(_{50}\) 13.4µM, IC\(_{50}\) 18.0µM, and IC\(_{50}\) 2.0µM, respectively; Figure 1). Interestingly, recent findings by Musarrat and colleagues showed that nelfinavir dramatically inhibits cell-to-cell fusion caused by the SARS-CoV-2 spike (S) glycoprotein.\(^6\) The inhibitory effect of nelfinavir on cathepsin L reported here may explain these findings and is in line with a critical role for cathepsin B/L reported previously.\(^2,3\) In summary, our findings describe a previously unknown inhibitory effect of the FDA-approved drugs saquinavir and nelfinavir on cathepsin L activity. Whether the pivotal role for cathepsin L in Sars-Cov-2 infection described in vitro can be translated to humans, our results support clinical trials of saquinavir or nelfinavir, preferentially combined with camostat as a potential treatment for COVID-19 patients.

Declarations

Author Contributions: Dr. Montenegro had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Montenegro, Tracey, and Billiar. The compound M374.1 was designed and synthesized by Al-Abed and He.

Acquisition, analysis, interpretation of data: Montenegro.

Drafting of the manuscript: Montenegro and Billiar.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Montenegro.

Conflict of interest statements: The authors declare no conflict.

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Ethics committee approval: Not applicable

References


Figures

**Figure 1**

Effects of HIV protease inhibitors on the activity of cathepsin L. The HIV protease inhibitors saquinavir and nelfinavir, saquinavir-based compound M374.1, and the protease inhibitor SID 26681509 inhibited human cathepsin L in a concentration-dependent manner ( Panels A, B, C, and D, respectively). The
cysteine protease inhibitor E-64 was used as a positive control. Data are shown as mean ± S.E.M. N=5-7 for each concentration. * P< 0.05 versus vehicle after Dunnett's multiple comparison test using one-way analysis of variance