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

Marcelo Freitas Montenegro (marcelofm@alumni.usp.br)
Department of Surgery, University of Pittsburgh, 200 Lothrop St, 15213, Pittsburgh, PA, USA.
https://orcid.org/0000-0001-9137-8145

Yousef Al-Abed
The Feinstein Institute for Medical Research, 350 Community Drive, 11030, Manhasset, NY, USA.

Mingzhu He
The Feinstein Institute for Medical Research, 350 Community Drive, 11030, Manhasset, NY, USA.

Kevin J. Tracey
The Feinstein Institute for Medical Research, 350 Community Drive, 11030, Manhasset, NY, USA.
https://orcid.org/0000-0003-1884-6314

Timothy R. Billiar (billiartr@upmc.edu)
Department of Surgery, University of Pittsburgh, 200 Lothrop St, 15213, Pittsburgh, PA, USA.

Short Report

Keywords: HIV protease inhibitors, Cathepsin L, Sars-Cov-2, COVID-19, Cathepsin L inhibitors

Posted Date: June 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-37258/v1

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.[1] 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.[2] 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.[2] 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[3] 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.[1] 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.[4] 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* IC_{50} 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.

Role of funding source: There is no founding source

Ethics committee approval: Not applicable

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Figures

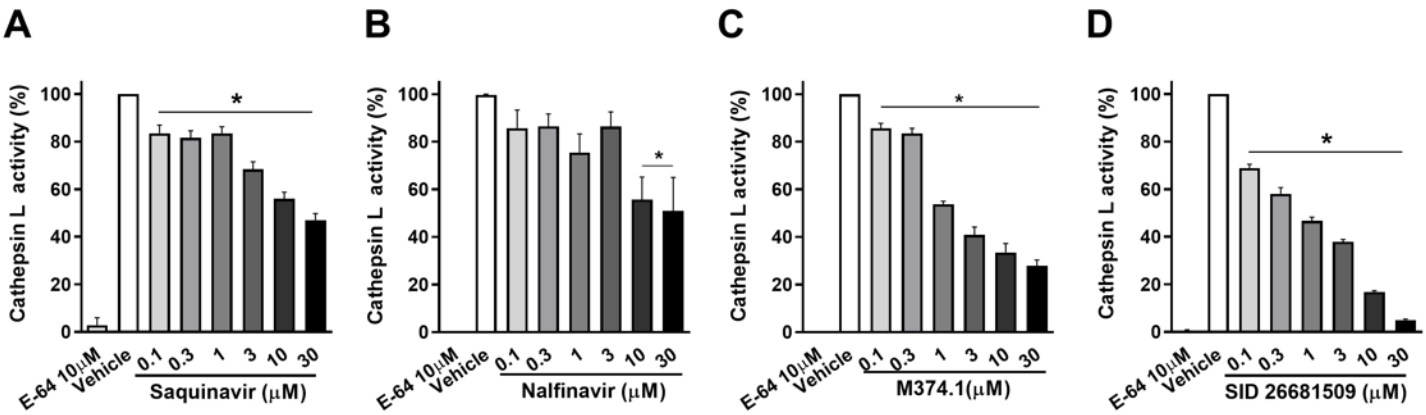

Figure 1

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