




Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19

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The currently unfolding coronavirus pandemic threatens health systems and economies worldwide. The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated disease coronavirus disease 2019 (COVID-19) has initially been limited to China. However, the virus has now been detected in more than 100 countries outside China, and major outbreaks are ongoing in the United States, Italy, and Spain. At present, our antiviral arsenal offers little protection against SARS-CoV-2, although recent progress has been reported (1), and novel antivirals are urgently needed to mitigate the COVID-19 health crisis.

The SARS-CoV-2 spike protein (S) is inserted into the viral envelope and mediates viral entry into cells. For this, the S protein depends on the cellular enzyme transmembrane protease serine 2 (TMPRSS2), which cleaves and thereby activates the S protein (2). SARS-CoV (3–5) and other coronaviruses (6, 7) also use TMPRSS2 for S protein activation, and the protease is expressed in SARS-CoV target cells throughout the human respiratory tract (8). Moreover, TMPRSS2 is required for spread of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) in rodent models (9, 10) but is dispensable for development and homeostasis in mice (11). Thus, TMPRSS2 constitutes an attractive drug target.

Recent work shows that camostat mesylate (NI-03), a serine protease inhibitor active against TMPRSS2 and employed for treatment of pancreatitis in Japan, inhibits SARS-CoV-2 infection of human lung cells (2). The suitability of camostat mesylate for treatment of COVID-19 is currently being evaluated in a clinical trial (12), but it is unclear whether compound concentrations can be attained in the lung that are sufficient to suppress viral spread. In the absence of this information, testing of other serine protease inhibitors for blockade of SARS-CoV-2 entry is an important task. For this, we tested gabexate mesylate (FOY) and nafamostat mesylate (Futhan) (13) along with camostat mesylate for inhibition of SARS-CoV-2 infection of lung cells. All compounds are approved for human use in Japan, and nafamostat mesylate inhibits TMPRSS2-dependent host cell entry of MERS-CoV (14). A comparison of the antiviral activities of the three compounds revealed that none interfered with cell viability or with host cell entry mediated by the glycoproteins of vesicular stomatitis virus or Machupo virus (Fig. 1A), which served as negative controls. Gabexate mesylate slightly inhibited SARS-CoV-2 S-driven host cell entry while camostat mesylate robustly suppressed entry (Fig. 1A). Notably, nafamostat mesylate, which is FDA approved for indications unrelated to coronavirus infection, inhibited SARS-CoV-2 S-mediated entry

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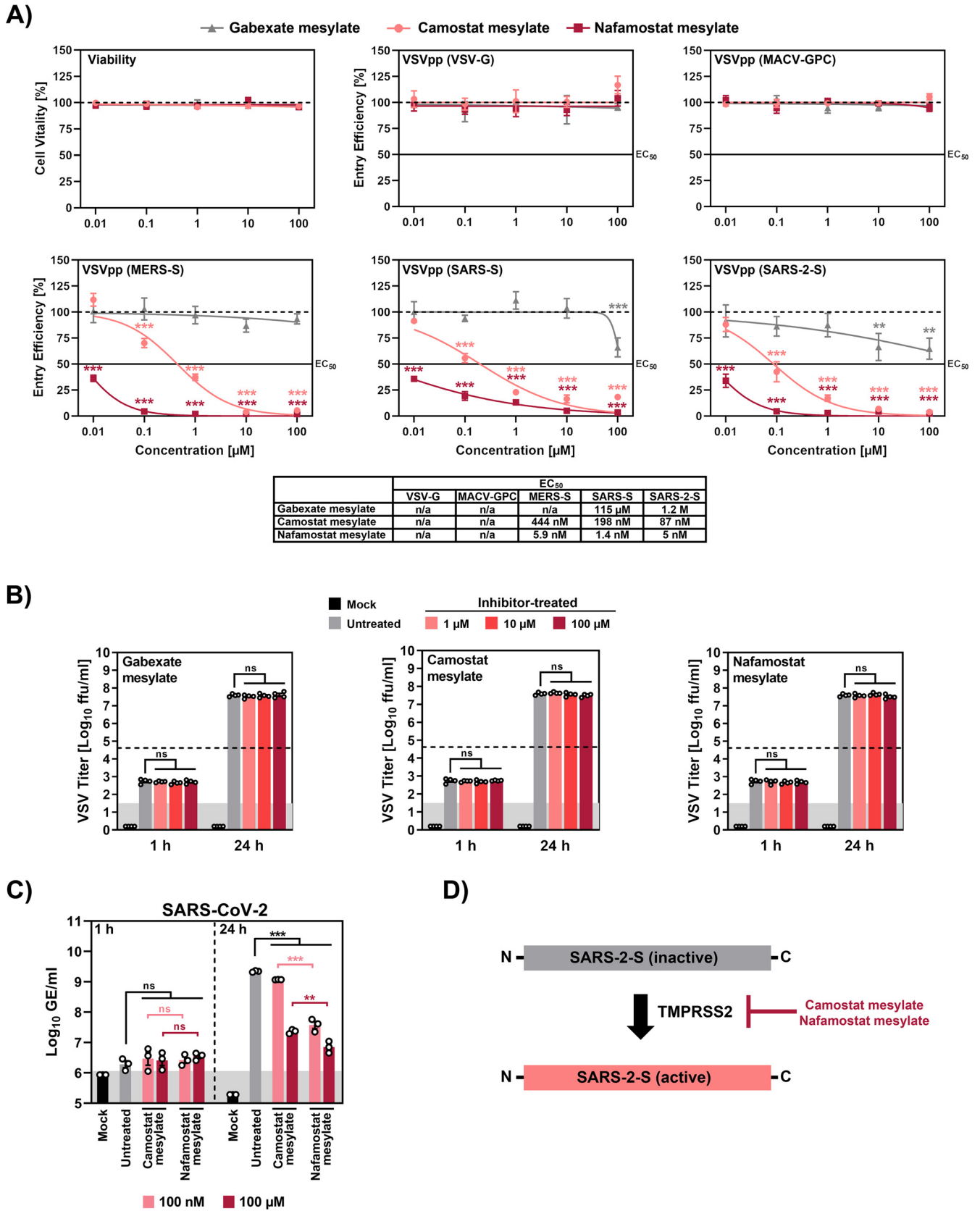


FIG 1 Nafamostat mesylate inhibits SARS-CoV-2 infection of lung cells in the nanomolar range. The lung-derived human cell line Calu-3 was incubated with the indicated concentrations of the indicated serine protease inhibitors, and (A) either cell viability was measured or the cells were inoculated with vesicular (Continued on next page)

FIG 1 Legend (Continued)

stomatitis virus reporter particles pseudotyped with the indicated viral glycoproteins. The efficiency of viral entry was determined at 16 h postinoculation by measuring luciferase activity in cell lysates. The 50% effective dose values are indicated below the graphs. In parallel, cells exposed to serine protease inhibitors were infected with replication-competent vesicular stomatitis virus encoding green fluorescent protein (B) or infected with SARS-CoV-2 (C), and infection efficiency was quantified by focus formation assay and by measuring genome copies via quantitative RT-PCR, respectively. A scheme of how camostat and nafamostat mesylate block activation of SARS-2-S is shown in panel D. The average from three independent experiments is shown in panels A and C while the average from four independent experiments is presented in panel B. For panels A to C, statistical significance was tested by two-way analysis of variance with Dunnett's posttest. In addition, statistical significance of differences between SARS-CoV-2 genome equivalents at identical concentrations of camostat or nafamostat mesylate was tested by one-way analysis of variance with Sidak's posttest. Abbreviations: VSV-G, vesicular stomatitis virus glycoprotein, MACV-GPC, Machupo virus glycoprotein complex; MERS-S, Middle East respiratory syndrome coronavirus spike glycoprotein; SARS-S, severe acute respiratory syndrome coronavirus spike glycoprotein; SARS-2-S, severe acute respiratory syndrome coronavirus 2 spike glycoprotein.

into host cells with roughly 15-fold-higher efficiency than camostat mesylate, with a 50% effective concentration [EC₅₀] in the low-nanomolar range (Fig. 1A). Moreover, nafamostat mesylate blocked SARS-CoV-2 infection of human lung cells with markedly higher efficiency than camostat mesylate while both compounds were not active against vesicular stomatitis virus infection, as expected (Fig. 1B to D). In light of the global impact of COVID-19 on human health, the proven safety of nafamostat mesylate, and its increased antiviral activity compared to camostat mesylate, we argue that this compound should be evaluated in clinical trials as a COVID-19 treatment.

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