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Anti-viral and Anti-inflammatory Effects of Camostat and Nafamostat on Influenza Virus and Coronavirus Infections in Human Airway Cells and the Mouse Lungs

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Abstract

Influenza viruses and coronaviruses cause several human diseases, such as bronchitis, bronchiolitis, and pneumonia, and exacerbate bronchial asthma, chronic obstructive pulmonary disease and pulmonary fibrosis. Human airway epithelial cells infected with these viruses release progeny viruses and inflammatory cytokines, such as Interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor-α, partly through the activation of nuclear factor kappa B. Modulation of airway damage and inflammation may modulate viral infection-induced airway and lung diseases. Human tracheal and nasal epithelial cells express proteases, including Transmembrane Protease Serine S1 Member 2 (TMPRSS2), and the proteases activate influenza viruses and coronaviruses and the subsequent replication processes of these viruses. The protease inhibitors camostat and nafamostat reduced influenza virus and coronavirus replication and the amounts of cytokines released from human airway epithelial cells. Nafamostat also reduced the release of influenza virus in the lungs of mice. The development of clinically available protease inhibitors is required to treat patients infected with influenza virus or coronavirus.

Keywords: Airway epithelial cells • Bronchial asthma • Coronavirus • Influenza virus • Transmembrane protease serine 2

Abbreviations: COPD: Chronic Obstructive Pulmonary Disease, CD13: aminopeptidase N, HAT: Human Trypsin-like Protease, HCoV: Human Coronavirus, HNE cell: Human Nasal Epithelial cell, HTE cell: Human Tracheal Epithelial cell, IL: Interleukin, TMPRSS2: Transmembrane Protease Serine S1 Member 2, TNF: Tumor Necrosis Factor, Vero E6 cell: African Green Monkey Kidney cell.

About the Study

Influenza viruses resistant to neuraminidase inhibitors and baloxavir have been identified, and several patients with influenza virus infection have died despite intensive drug treatment. Anti-viral drugs against seasonal coronaviruses have not been developed. Furthermore, because of the worldwide outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, effective drugs are needed to treat patients with Coronavirus Disease 2019 (COVID-19), even though some drugs have already been developed. Therefore, further development of anti-influenza and anti-coronavirus drugs is needed. Influenza viruses and coronaviruses are activated by proteases, including Transmembrane Protease Serine S1 Member (TMPRSS) 2 expressed in airway epithelial cells [1]. Here, we report the findings of our studies on the effects of the protease inhibitors camostat and nafamostat on the proliferation of influenza viruses and seasonal coronaviruses and on the viral infection-induced production of inflammatory cytokines.

Effects of protease inhibitors on influenza virus infection

Infection with influenza virus induces bronchitis, pneumonia and pulmonary fibrosis and exacerbates bronchial asthma and Chronic Obstructive Pulmonary Disease (COPD). We identified that the TMPRSS2

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and Human Trypsin-like Protease (HAT; TMPRSS11D), which are known to cleave HA0 and activate influenza virus [2], were expressed at the cell membrane and in the cytoplasm of cultured Human Tracheal Epithelial (HTE) cells, and mRNA transcripts encoding TMPRSS2, TMPRSS4 and TMPRSS11D were detectable in these cells (Table 1) [3]. The protease inhibitor camostat, which has been used for the treatment of pancreatitis, reduced the amounts of pandemic (A/Sendai-H/ 108/2009/(H1N1) pdm09) and seasonal (A/ New York/ 55/2004(H3N2)) type A influenza virus strains in the supernatant and/or viral RNA in cells (Table 1) [3]. Camostat reduced the cleavage of the influenza virus precursor protein HA0 into the subunit HA1. Camostat also reduced the concentrations of Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-α in the supernatant. The mRNA expression levels of TMPRSS2, TMPRSS4 and TMPRSS11D were not affected by camostat. Other types of protease inhibitors, including gabexate and aprotinin, also reduced the viral titers and RNA levels in HTE cells, and aprotinin reduced the concentration of TNF-α in the supernatant. These findings suggest that the clinically used protease inhibitor camostat may reduce influenza virus replication and infection-induced airway inflammation.

Pretreatment with nafamostat, which has been used for the treatment of disseminated intravascular coagulation, reduced viral RNA levels in primary HTE and Human Nasal Epithelial (HNE) cells and the titers of pandemic (A/Sendai-H/108/2009/(H1N1) pdm09) and seasonal (A/New York/55/2004(H3N2)) type A influenza virus strains; it also reduced the secretion of inflammatory cytokines, including IL-6 and TNF-α, into the supernatants of cells infected with the pandemic influenza virus strain (Table 1) [4]. HNE and HTE cells exhibited mRNA and/or protein expression of TMPRSS2, TMPRSS4, and TMPRSS11D. Pretreatment with nafamostat reduced the cleavage of the precursor protein HA0 of the pandemic influenza virus strain into the subunit HA1 in HTE cells and reduced the number of acidic endosomes in both HTE and HNE cells in which influenza virus RNA enters the cytoplasm. Additionally, nafamostat (30 mg/kg/day, intraperitoneal administration) reduced the levels of the pandemic influenza virus strain (A/Hyogo/YS/2011 (H1N1) pdm09) in mouse lung washes. These findings suggest that nafamostat may also inhibit influenza virus replication in human airway epithelial cells and the mouse lungs, and reduce infection-induced airway inflammation by modulating cytokine production.

Table 1: Effects of camostat and nafamostat on influenza virus and seasonal coronavirus replication and cytokine production in human airway epithelial cells and the mouse lungs.

Drugs/Viruses	Camostat	Ref No.	Nafamostat	Ref No.
Influenza virus	Reductions in	[3]	Reductions in	[4]
	1) The release of pandemic and seasonal influenza virus strains		1) The release of pandemic and seasonal virus strains	
	2) The replication of the viral RNA of pandemic and seasonal influenza virus strains		2) The replication of the viral RNA of pandemic and seasonal influenza virus strains	
	3) The cleavage of an influenza precursor protein		3) The cleavage of an influenza precursor protein	
	4) The viral infection-induced production of IL-6 and TNF-α in HTE cells.		4) The number of acidic endosomes	
	5) No effects on TMPRSS2 mRNA expression in HTE cells.		5) The viral infection-induced production of IL-6 and TNF-α in HTE and/or HNE cells.	
			6) No effects on TMPRSS2 mRNA expression in HTE or HNE cells.	
			Reductions in the release of the pandemic influenza virus into mouse lung washes.	
HCoV-229E	Reductions in the release of HCoV-229E into the airway surface fluid of HNE cells.	[5]	Reductions in the release of HCoV-229E into the airway surface fluid of HNE cells.	[5]

Effects of protease inhibitors on coronavirus infection

Human seasonal coronaviruses, including Human Coronavirus (HCoV)-229E, HCoV-OC43, and HCoV-HKU1, also cause the common cold, bronchitis, and pneumonia and exacerbate bronchial asthma and COPD. HCoV-229E binds to the aminopeptidase N (CD13) receptor and enters cells partly via cell-surface pathways using TMPRSS2 [1]. To examine the effects of protease inhibitors on the replication of HCoV-229E, we pretreated primary cultures of HNE cells with camostat or nafamostat and then infected the cells with HCoV-229E; the viral titers in the Airway Surface Liquid (ASL) of the cells were examined. Pretreatment with camostat or nafamostat reduced the titers of HCoV-229E (Table 1) [5] in the ASL of cultured HNE cells. Furthermore, a significant amount of the TMPRSS2 protein was detected in the ASL of HNE cells. These findings suggest that the protease inhibitors camostat and nafamostat may also reduce HCoV-229E replication in human airway epithelial cells.

Conclusion

We demonstrated that camostat and nafamostat reduced the proliferation of influenza viruses and a seasonal coronavirus in human airway cells and the mouse lungs. Although the effects of the protease inhibitors are still uncertain on the proliferation of influenza viruses which are resistant to neuraminidase inhibitors and baloxavir. Wang, et al. also demonstrated that nafamostat reduced SARS-CoV-2 replication by inhibiting TMPRSS2 in African Green Monkey Kidney (Vero E6) cells [6]. These findings suggest that SARS-CoV-2 enters partly *via* cell-surface pathways using TMPRSS2. In contrast to the effects observed in the *in vitro* studies, the clinical efficacy of nafamostat in the treatment of COVID-19 is still uncertain, and anti-viral drugs for influenza viruses and seasonal coronaviruses have not been developed. Thus, new types of protease inhibitors without adverse effects are expected to be developed.

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Conflict of Interest

The authors have no conflicts of interest.

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