

Simultaneous Treatment of COVID-19 With Serine Protease Inhibitor Camostat and/or Cathepsin L Inhibitor?

Stefan Bittmann^{a, b}, Anne Weissenstein^a, Gloria Villalon^a,
Elena Moschuring-Alieva^a, Elisabeth Luchter^a

To the Editor

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin II receptor in different tissues of the body, especially in the mouth cavity and the tongue and the skin [1, 2]. Therefore, it needs the serine protease, transmembrane serine protease 2 (TMPRSS2), for cell entry [2]. Cathepsin L/B (CTSL/CTSB) plays an important role in a second pathway, the endosomal pathway.

SARS-CoV-2 binds to angiotensin II receptor (angiotensin converting enzyme 2 (ACE-2))

Based on the newest results of the research, the new coronavirus uses the protein ACE-2 as a receptor for docking to pneumocyte type II [2]. A broadly put up team under the direction of infection biologists of the German Primate Centre and with participation of the Charite Berlin, the Endowment Veterinary College of Hannover, the Accident Clinic BG Murnau which wanted to find out Munich LMU, the Robert of Koch's Institute and the German Centre for Infection researched how SARS-CoV-2 penetrates into lung cells and how this process can be blocked [3]. They have published their results in the magazine *Cell* [3]. They also identified the TMPRSS2 as responsible cellular protein for the entry into the cell [3]. The type II transmembrane protease pro-TMPRSS2 activates the spike (S) protein of SARS-CoV on the cell surface after the recipient, which binds in cells during virus access. Without TMPRSS2, SARS-CoV-2 reaches cell access via an endosomal pathway, in which CTSL can play an important role with the activation of the S protein fusogenicity.

SARS-CoV-2 needs for cell entry ACE-2, TMPRSS2 and CTSL

Substantially for the entry, the control of S protein of the virus

is by presence of serine protease TMPRSS2 and CTSL. TMPRSS2 activates S protein for cell-cell and virus-cell fusion in transformation only. The S protein is fusogenetically activated by CTSL, therefore allowing fusion of the viral and endosomal membranes. Subsequent infection is sensitive to inhibitors of endosomal acidification such as ammonium chloride, suggesting that SARS-CoV-2 requires a low-pH milieu for infection. On the other hand, S protein can mediate cell-cell fusion at neutral pH, indicating that S protein-mediated fusion does not include an absolute requirement for an acidic environment. Given these discordant findings, we hypothesized that cellular factors sensitive to ammonium chloride, such as pH-dependent endosomal proteins, may play a role in mediating SARS-CoV-2 viral entry. The requirements for proteases in the activation of viral infectivity and the effect of protease inhibitors on COVID-19 infection are examined. Our results are consistent with a model in which SARS-CoV-2 employs a unique three-step method for membrane fusion, involving receptor binding and induced conformational changes in S glycoprotein followed by CTSL proteolysis and activation of membrane fusion within endosomes.

TMPRSS2

TMPRSS2 encodes a protein that belongs to the serine protease family. The encoded protein contains a type I transmembrane area, a receiver classified one area, a garbage collector receiver cysteine-rich area and a pro-sweep area. Serine proteases are known to be involved in many physiological and pathological processes. This gene was demonstrated to be regulated by androgenic hormones in prostate cancer cells and regulated in androgen-independent prostate cancer tissue. It is thought that the pro-inflammatory area of this protein is cleaved and hidden in the cell media after auto splitting. Alternately split transcript variants that encode different isoforms have been found for this gene.

Camostat

Camostat (mesylate) is supplied as a crystalline solid. A stock solution can be prepared by dissolving the camostat (mesylate) in the solvent of choice, which should be purged with an inert gas. The camostat (mesylate) is soluble in organic solvents such as dimethyl sulfoxide (DMSO) and dimethylformamide.

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^aDepartment of Pediatrics, Ped Mind Institute (PMI), Gronau, Germany

^bCorresponding Author: Stefan Bittmann, Department of Pediatrics, Ped Mind Institute (PMI), Hindenburgring 4, D-48599 Gronau, Germany.

Email: stefanbittmann@gmx.de

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Table 1. Different Therapeutic Approaches to Treat COVID-19

Classes	Treatment option	
Anti-viral drug	> 85% of patients received antiviral drugs, including oseltamivir (75 mg every 12 h orally), ganciclovir (0.25 g every 12 h intravenously) and lopinavir/ritonavir tablets (400/100 mg twice daily); lopinavir/ritonavir sirup in children; remdesivir is currently in trials in more than 10 medical facilities in Wuhan and is known to prevent MERS-CoV-2	In trials
	Ribonucleoside analog NHC (EIDD-1931) [10]	<i>In vitro</i>
Anti-malarial drug	Chloroquin and hydroxychloroquin have been effective in inhibiting the exacerbation of pneumonia due to the anti-viral and anti-inflammatory activities	Controversial
Herbal agent	During the last SARS-CoV outbreak, traditional Chinese medicine was widespread and is currently used in China. The five most commonly used herbs were Astragali Radix (Huangqi), Glycyrrhizae Radix and Rhizoma (Gancao), Saposhnikoviae Radix (Fangfeng), Atractylodis Macrocephalae Rhizoma (Baizhu) and Loniceræ Japonicæ Flo	Add-on therapy
TMPRSS2 inhibitor	Camostat [3, 8, 9], nefamostat [9]	First trials started; University of Aarhus and Tokyo; possible inhalative application?
CD147 inhibitor	Meplazumab [7]	Add-on therapy [7]
MC antibody treatment/ mesenchymal stem cell TX	Monoclonal antibodies against S protein, against ACE-2, against viral protein	In trials

NHC: β -D-N4-hydroxycytidine; TMPRSS2: transmembrane serine protease 2; ACE-2: angiotensin converting enzyme 2; MERS-CoV: Middle East respiratory syndrome coronavirus; SARS-CoV: severe acute respiratory syndrome coronavirus.

The solubility of camostat (mesylate) in these solvents is about 25 mg/mL. Camostat is a protease inhibitor [3, 4]. It inhibits trypsin ($K_i = 1$ nM) and various inflammatory proteases, including plasmin, kallikrein and thrombin. Camostat inhibits the incorporation of the SARS-CoV and the surface glycoprotein COVID-19 into pseudotyped particles of vesicular stomatitis virus (VSV) in Vero cells, Calu-3 cells and primary human lung epithelial cells when administered at a concentration of 10 μ M [4]. It reduces the number of genomic equivalents of SARS-CoV-2, a marker of infection, in Calu-3 cells. Camostat inhibits the function of the sodium channel in human respiratory epithelial cells ($IC_{50} = 50$ nM) and improves mucociliary clearance in sheep [4]. Administration of camostat (1 mg/kg) inhibits the production of tumor necrosis factor- α (TNF- α) and monocyte chemoattractant protein-1 by monocytes and the proliferation of pancreatic star cells in a rat model of pancreatic fibrosis [2]. Camostat is in Japan well known as “Foipan”. In Japan, camostat is certified for patients with chronic pancreatitis and showed attenuating effect in pancreatic fibrosis [5]. For SARS-CoV-2 the first clinical trials were initiated at the University of Aarhus, Denmark. Camostat has the potential to block the entry of the virus into the lung cells, well known as pneumocytes type 2. To date, no clinical studies were performed, nor any results are present. What we know is that camostat could have a promising potential in COVID-19.

CTSL inhibitor

SARS-CoV-1 used CTSL, and infection could be blocked by

CTSL inhibitors in mouse model [6]. SARS-CoV-2 could be blocked in a similar way; therefore, clinical trials should be performed as soon as possible with CTSL inhibitors in COVID-19.

Conclusions

To date, many different treatment options are used “off-label” in severe cases or are under clinical trial. Table 1 shows the different therapeutic approaches to treat COVID-19. In this work, we focus on serine protease inhibitor camostat, which partially blocks infection by SARS-CoV-2 [2, 3, 7-10]. Simultaneous treatment of the cells with camostat and a cathepsin inhibitor has efficiently prevented both cell access and multi-step growth of SARS-CoV-2 in human Calu-3 weather pathway epithelial cells [11]. This efficient inhibition could be attributed to the double blockade of access from the cell surface and through the endosomal pathway. These observations suggest camostat as a candidate antiviral drug to prevent or suppress TMPRSS2-dependent infection by the SARS-CoV-2 [8, 9]. Further investigations are necessary. A new therapy option in COVID-19 patients is based on the combination of camostat (serine protease TMPRSS2 inhibitor) and a cathepsin inhibitor [5, 6]. First clinical off-label trials should be performed as soon as possible in COVID-19 patients. In a recent study, an orally bioavailable broad-spectrum antiviral, ribonucleoside analog β -D-N4-hydroxycytidine (NHC, EIDD-1931), showed positive results of inhibiting SARS-CoV-2 in human airway epithelia cell cultures and multiple coronaviruses in mice [10]. Further trials

pointed out the role of a new receptor in COVID-19 pathology, the CD147 receptor (basigin, EMMRIN). CD 147 receptor can be blocked by meplazumab as add-on therapy and needs further prospective trials to classify the role in COVID-19 treatment, especially in cytokine release syndrome [7, 12].

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Conflict of Interest

None to declare.

Informed Consent

Not applicable.

Author Contributions

All authors contributed to this work and proved the available data and final manuscript.

Data Availability

The authors declare that data supporting the findings of this study are available within the article.

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