

## ***Blocking Serine Protease (TMPRSS2) by Bromhexine; looking at potential treatment to prevent COVID-19 Infection***

**Ramin Tolouian, MD<sup>1</sup>, Audrey Tolouian, EdD, MSN, RN-CNE<sup>2</sup>,  
Mohammadreza Ardalan, MD<sup>3</sup>**

### **ABSTRACT**

Coronaviruses have caused at least three large scale epidemics/pandemics in humans over the past twenty years – severe acute respiratory syndrome (SARS) in 2003; the Middle East respiratory syndrome (MERS) in 2012; novel coronavirus disease (COVID-19) in 2019 – taking many lives all over the world. World Health Organization (WHO) named the new virus responsible for COVID-19 as SARS-CoV-2.

### **KEYWORDS**

Serine protease, TMPRSS2, COVID-19, Bromhexine, Virus priming, Coronavirus Treatment, Prevention, Rural Area

*Author affiliations are listed at the end of this article.*

*Correspondence to:*  
Mohammadreza Ardalan, MD  
Tabriz University of  
Medical Sciences  
[ardalanm@tbzmed.ac.ir](mailto:ardalanm@tbzmed.ac.ir)

### **INTRODUCTION**

Coronaviruses have caused at least three large scale epidemics/pandemics in humans over the past twenty years – severe acute respiratory syndrome (SARS) in 2003; the Middle East respiratory syndrome (MERS) in 2012; novel coronavirus disease (COVID-19) in 2019 – taking many lives all over the world. World Health Organization (WHO) named the new virus responsible for COVID-19 as SARS-CoV-2.

For the SARS outbreak, the case-fatality rate was 11% while for MERS it was 34%.<sup>1</sup> According to Johns Hopkins University Coronavirus Resource Center, the case-fatality rate for COVID-19 pandemic varies in reports from different countries. The mortality rate in the top ten most affected countries worldwide is between 4.5-16.3%. This virus is highly contagious and is responsible for taking more than 320,000 lives and affecting around 5 million people around the world, as of mid-May 2020. The mortality is almost twice higher in males compared to females.<sup>2</sup> The White House Coronavirus Task Force estimated there will be between 100,000-240,000 deaths due to COVID-19 in the US alone. Despite the implementation of many preventive policies all

over the world i.e. social distancing, wearing masks etc., this virus is continuing to kill people, has taxed community health care centers, and therapeutic options are currently limited.<sup>3</sup>

There is an urgent need to better understand the pathophysiologic mechanism of the virus life cycle and further, find a way to prevent its fatal complications.

### **VIRUS CYCLE**

This virus has a strong affinity toward angiotensin-converting enzyme 2 (ACE2) receptors in the respiratory system via S Protein on its envelope. S Protein is composed of one amino-terminal (S1) and one carboxy-terminal (S2). Cleavage at S1-S2 junction by protease is essential to activate membrane fusion. After binding, there are two different pathways through which the virus can enter the cell. First is the endosomal pathway which allows the virus entry at the plasma membrane. In the endosomal pathway, cathepsin [Cysteine protease] is responsible for the activation of membrane fusion and endocytosis. Low pH facilitates the endosomal cell entry in this path. Second is the non-endosomal pathway



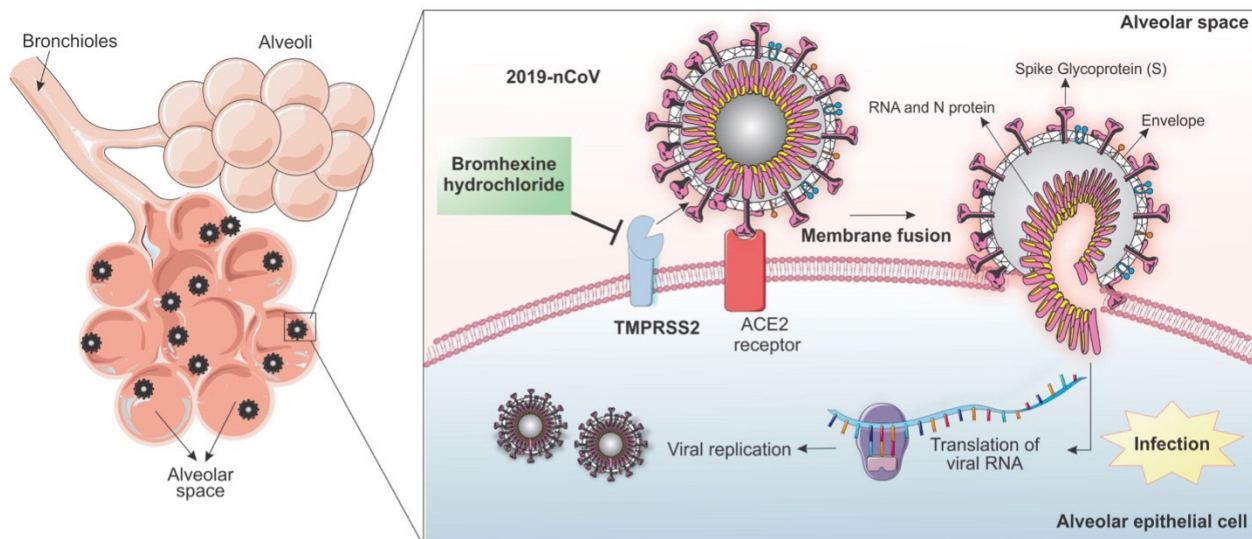


FIGURE 1: COVID-19 cell entry via the Non-Endosomal pathway.

which allows the virus entry into the cell without endocytosis. In this pathway, TMPRSS2 [Serine protease] is needed to prime the spikes and allow the virus entry at the plasma membrane. After the virus enters the cell, in the next phase, the virus's RNA replicates and eventually causes cell death. After cell death, the virus continues this cycle and infects other cells.<sup>4,5</sup> (Figure 1)

A host serine protease (TMPRSS2) is responsible for priming the spikes and allows the virus entry at the plasma membrane via the non-endosomal pathway. Cleavage at S1-S2 junction by TMPRSS2 activates membrane fusion. Bromhexine hydrochloride is shown to act as a bioavailable inhibitor of TMPRSS2.

## THERAPEUTIC OPTIONS

### ENDOCYTOSIS INHIBITORS

Many medications have been used to interfere and block the virus cycle with different results. It has been proposed that chloroquine, by increasing the endosomal pH, may abrogate virus-endosomal fusion and endocytosis, in addition to possibly exerting an antiviral effect inside the endosome. There is limited promising data about the efficacy of chloroquine or hydroxychloroquine in COVID-19. Amiodarone might be another effective agent in blocking endocytosis, but no solid data is yet available to show its efficacy.<sup>6</sup>

### TMPRSS2 INHIBITORS

Blocking the non-endosomal pathway might be an effective option to control COVID infection. The serine protease (TMPRSS2) could be a good target to prevent viral infection by blocking the fusion and priming the processes of the virus.<sup>5</sup> There are some medications such as camostat mesylate or nafamostat, which are used in chronic pancreatitis to inhibit the TMPRSS2 and eventually cell entry of SARS-COV-2. A trial using nafamostat is ongoing. No data is available now.<sup>6</sup>

TMPRSS2 is a member of the type II transmembrane serine protease (TTSP) family. TTSP is expressed in the GI system, lungs, kidneys, and prostate. It has been shown that the presence of serine protease of TMPRSS2 is very essential for the influenza virus infection. This enzyme cleaves the surface glycoprotein hemagglutinin (HA) of the influenza virus and ensues virus fusion and propagation.<sup>7</sup> The inhibition of TMPRSS2 by bromhexine can prevent influenza infection.<sup>8</sup> In addition to bromhexine, other synthetic inhibitors of TMPRSS2 have been developed and have demonstrated efficacy in preventing influenza infection. However, these inhibitors also have strong affinities for other proteases, such as matriptase, making their precise therapeutic mechanism unclear.<sup>9</sup>

TMPRSS2 expression in the prostate is driven by androgen receptor signaling. TMPRSS2 mRNA expression is upregulated and remains elevated in androgen-stimulated prostate cancer. The administration of bromhexine hydrochloride, an inhibitor of TMPRSS2, was able to suppress distant metastasis to the liver and lungs sites in mice models via modulating of ERG oncogene.<sup>10</sup> This link between androgen receptor signaling and TMPRSS2 expression could explain the higher prevalence and severity of coronavirus in males as compared to females.<sup>11</sup>

## OTHERS

Many antivirals, HIV medications, and steroids have been tried with no promising results in COVID-19, except limited clinical improvement in compassionate use of remdesivir which led to an emergency approval of medication by FDA but a multicenter, randomized, double-blind, placebo-controlled study did not show any significant clinical benefit.<sup>12,13</sup>

There is a debate about the role of angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARB) in COVID-19. The data showed that increasing the number of ACE2 receptors may not translate, as a rule, to getting the COVID-19 infection. It seems ACE2 receptor in conjunction with TMPRSS2, is a recipe for getting the infection.<sup>6</sup>

## RECOMMENDATION

Many different medications have been tried to prevent, control or treat COVID-19 with debatable results. Bromhexine hydrochloride is an inexpensive and safe over-the-counter medication that has been used as a mucolytic since 1963 in clinical medicine. It increases the secretion of various mucous components and improves mucociliary clearance.<sup>14</sup> This medication can also inhibit TMPRSS2 and potentially prevent priming of the S-protein of coronavirus and abort the process of the virus entry into the cell.<sup>15</sup>

This idea has major public health importance and could change clinical practice or provide a novel approach to COVID-19 infection for clinicians,

especially in underserved and rural areas with limited access to tertiary medical centers. The authors propose to use bromhexine 8 mg two or three times a day as a prophylactic/therapeutic measure during the COVID-19 pandemic. This monotherapy is safe and very inexpensive and therefore, most everybody can afford to get it. Further studies and clinical trials are needed to prove the validity of this medication therapy in COVID-19 infection. Multiple trials for bromhexine alone or in combination with hydroxychloroquine have been submitted but no results have yet been available.

## AUTHOR AFFILIATIONS

1. Division of Nephrology, University of Arizona, Tucson, Arizona, USA
2. School of Nursing, The University of Texas at El Paso, El Paso, Texas, USA
3. Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

## REFERENCES

1. Alghamdi IG, Hussain II, Almalki SS, Alghamdi MS, Alghamdi MM, El-Sheemy MA. The pattern of Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive epidemiological analysis of data from the Saudi Ministry of Health. *Int J Gen Med.* 2014;7:417-23.
2. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, et al. Gender differences in patients with COVID-19: focus on severity and mortality. *Front Public Health.* 2020;8:152.
3. Hamidian JA, Mazloom S, Ballard D. What the European and American health care systems can learn from China COVID-19 epidemic; action planning using purpose designed medical telecommunication, courier services, home-based quarantine, and COVID-19 walk-in-centers. *Immunopathol Persa*2020. p.e17.
4. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses - drug discovery and therapeutic options. *Nat Rev Drug Discov.* 2016;15(5):327-47.
5. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor.



- Cell. 2020.
6. Perico L, Benigni A, Remuzzi G. Should COVID-19 concern nephrologists? Why and to what Extent? The emerging impasse of angiotensin blockade. *Nephron*. 2020;1-9.
  7. Laporte M, Naesens L. Airway proteases: an emerging drug target for influenza and other respiratory virus infections. *Curr Opin Virol*. 2017;24:16-24.
  8. Garten W, Braden C, Arendt A, Peitsch C, Baron J, Lu Y, et al. Influenza virus activating host proteases: Identification, localization and inhibitors as potential therapeutics. *Eur J Cell Biol*. 2015;94(7-9):375-83.
  9. Meyer D, Sielaff F, Hammami M, Böttcher-Friebertshäuser E, Garten W, Steinmetzer T. Identification of the first synthetic inhibitors of the type II transmembrane serine protease TMPRSS2 suitable for inhibition of influenza virus activation. *Biochem J*. 2013;452(2):331-43.
  10. Murray AS, Varela FA, List K. Type II transmembrane serine proteases as potential targets for cancer therapy. *Biol Chem*. 2016;397(9):815-26.
  11. Zununi Vahed S, Ghiyasvand S, Tolouian R, Noshad H, Tolouian A, Mohajel shoja M, et al. The footprint of androgen sensitive serine protease (TMPRSS2) in gender mortality with COVID-19. *Immunopathol Persa*. 2020;6(2).
  12. Grein J, Myers RP, Brainard D. Compassionate use of remdesivir in Covid-19. Reply. *N Engl J Med*. 2020;382.
  13. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2020;395(10236):1569-78.
  14. Zanasi A, Mazzolini M, Kantar A. A reappraisal of the mucoactive activity and clinical efficacy of bromhexine. *Multidiscip Respir Med*. 2017;12:7.
  15. Habtemariam S, Nabavi SF, Ghavami S, Cismaru CA, Berindan-Neagoe I, Nabavi SM. Possible use of the mucolytic drug, bromhexine hydrochloride, as a prophylactic agent against SARS-CoV-2 infection based on its action on the transmembrane serine protease 2. *Pharmacol Res*. 2020;157:104853.

