Ars Pharmaceutica

Candidatos potenciales farmacológicos inhibidores de *proteasa transmembrana de serina 2* en el tratamiento de *2019-ncov.*

Potential pharmacological candidates *Transmembrane protease, serine 2* inhibitors *2019-ncov* treatment.

Alfredo Montes[1,3], Snaider Montes-Ucros[2], Eduardo Arrieta[2]

1 Grupo GINUMED. Corporación Universitaria Rafael Núñez, Facultad Ciencias de la Salud, Programa de Medicina, Cartagena, Colombia.

2 Estudiante Programa de Medicina, Corporación Universitaria Rafael Núñez, Cartagena, Colombia.

3 Grupo de Investigación Microbiología Clínica y Ambiental, Universidad de Cartagena, Facultad de Ciencias Exactas y Naturales, Programa de Biología, Cartagena, Colombia.

http://dx.doi.org/10.30827/ars.v61i4.15688

Notas clínicas
Clinical Notes

Correspondence

Alfredo Montes
alfredo.montes@curnvirtual.edu.co

Acknowledgement

All the authors are grateful to Yorelis Güette for his valuable contribution and revision of the grammar of the paper, they also appreciate the Rafael Núñez University Corporation and University of Cartagena (Cartagena, Colombia) for the continuous support of his research.

Received: 17.07.2020
Accepted: 12.08.2020

RESUMEN

Introducción: La Enfermedad por coronavirus 2019 (COVID-19) causada por el virus SARS-CoV-2, con característica de infectar el tracto respiratorio causando un síndrome respiratorio agudo como paso inicial para ingresar a la célula huésped el virus usa los receptores ACE II y la proteína transmembrana TMPRSS2 para causar la infección, Por lo que se ha descrito diferentes tipos de fármacos para realizar su inhibición en la adhesión del paso inicial.

Metodología: Revisión no sistemática de artículos con la ayuda de palabras clave preestablecidas.

Resultados: En esta revisión presentaremos fármacos que inhiben este tipo de receptor, por lo tanto, estos medicamentos podrían considerarse candidatos potenciales para mitigar la propagación del SARS-CoV-2.

Palabras Clave: TMPRSS2; ACE II; COVID-19; 2019-nCoV; *Coronaviridae.*

ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus with characteristic of infecting the respiratory tract, causing severe acute respiratory syndrome. The virus uses the ACE II receptors and the transmembrane protein TMPRSS2 initial step to enter the host cell, this contribution described different types of drug, to perform its inhibition in initial step adhesion.

Methodology: Non-systematic review of articles with the help of preset keywords

Results: In this review we will present drugs that inhibitors of this type of receptor therefore these drugs could be considered potential candidates to mitigate the spread of SARS-CoV-2.

Keywords: TMPRSS2; ACE II; COVID-19; 2019-nCoV; *Coronaviridae.*

INTRODUCTION

The 2019 coronavirus disease epidemic (COVID-19) caused by the new SARS-CoV-2 virus has led to the spread of serious respiratory lung disease worldwide. According to Baltimore, this new virus belonging to the *Coronaviridae* family classified in group III, defined as a single-stranded RNA virus with a positive sense. It presents a generally large genome which could present mutations over time[1,2]. Several viruses in this family have been considered as infectious agents causing common colds, that is way they are in constant circulation in the human population. However, different viruses belonging to this family have appeared that have caused serious problems infecting thousands of people, such as (SARS-CoV) associated with Severe Acute Respiratory Syndrome in 2003, and (MERS-CoV)[3] associated with Middle East Respiratory Syndrome in 2002.

Currently (July, 16th 2020) the new coronavirus has spread in 188 countries which has infected 13,744,743 people causing 588,383 deaths[4] until today. The spread of this virus has increased significantly due to the type of transmission which has not yet reached a clear consensus; It has been said that this kind of virus has different forms of transmission: from animals to humans; from humans to animals; and from humans to humans; through contact; and respiratory droplets[5] . Furthermore, it is not certain that the virus is transmitted by air, however, the COVID-19 virus can be spread indirectly by contact with surfaces in its immediate environment or with objects that have been used by someone infected, highlighting that the viral particle can be stay on these surfaces for an estimated time[6].

Based on the above, the specific route of transmission of natural reservoirs to humans continues in contradictions. The most accurate evidence believed so far is that pangolins are responsible for providing this transmission to humans. This shows us that the receptor-binding domain of the S protein of pangolin-CoV is almost similar to SARS-CoV-2[7]. Different studies have shown that the angiotensin-converting enzyme 2 (ACE II) plays an important role as a receptor for SARS-CoV-2 in the binding of eukaryotic animal cells in the vicinity to infect, however, it has been intensified that the serine TMPRSS2 protease as a crucial adjuvant (cofactor) for the binding of this virus peak protein[8] converting these receptors as potential candidates for the development of white molecules that prevent the entry of the virus and thus counteract the pandemic caused by SARS-CoV-2. Therefore, this article identifies some receptor inhibitor molecules that contribute to virus adhesion in human host cells.

MATERIAL AND METHODS

A non-systematic review of clinical cases, original and review articles was carried out in indexed databases such as PubMed; ScienceDirect; Springer; and OVID in first, and second language from 2005-2020 mainly. For this research paper, The MeSH terms used for the article search and selection process were TMPRSS2, ACE II, COVID-19, 2019-nCoV, New treatment strategies, SARS-CoV-2, and *Coronaviridae*. Only the articles that had the MeSH terms were selected. This search strategy consisted of combining the MeSH terms as exclusion or inclusion criteria to make the search process more efficiently.

RESULTS AND DISCUSSION

The TMPRSS2 receptor has been studied as one of the main receptors for target cells with the assistance of ACE II. For this reason, different drugs that inhibit these two receptors have been tested, in order to counteract the adherence SARS-Cov-2 in human host cells.

Camostat mesilate and Nafamostat

Nowadays, Camostat has a wide range of uses. That is why this drug could be considered as one of the most promising candidates for the treatment of respiratory infections caused by SARS-Cov-2 today. Since this drug achieves the blocking of serine protease enzymes. In fact, this inhibitor was approved in Japan as a treatment of chronic pancreatitis, and postoperative reflux esophagitis [9]. According to studies, and clinical trials, Camostat can block the entry of the SARS-CoV2 virus inside the target cell. This has been demonstrated by in vitro cell test where this drug obstructs the adherence of the SARS-CoV virus, and the SARS-CoV-2 glycoprotein, if it is provided at a concentration of 10 μM[10]. On the other hand, different research suggests Nafamostat, a serine protease inhibitor, as another candidate drug for combating Covid-19, since it is known that Nafamostat blocks MERS-CoV virus infection in vitro by inhabiting the activity of TMPRSS2 thus achieving a 100-fold reduction in viral entry at a concentration as low as 1 nM. This means that it is more effective than Camostat. That said, Nafasmostat could contribute to blocking the entry of the new coronavirus into cells close to infecting it. This is how Nafamostat becomes another interesting drug for studies against SARS-COV-2[11].

Bromhexine Chlorhydrate

It is a mucolytic agent used in the treatment of respiratory disorders associated with excessive and viscous mucus. It

was previously used for the treatment of influenza virus, and coronavirus infections as an inhibitor of TMPRSS2. Bromhexim contributed significantly to the inhibition of trypsin-like proteases, expressed in the human respiratory tract of influenza, such as the SARS-CoV and MERS viruses[12].

According to the reports presented by Shen et *al*[13], this drug could be used for treatment of infections caused by viruses of the coronaviridae family that cause severe respiratory infections. They suggest that these drugs can be used to treat patients who are asymptomatic of covid-19, in order to prevent the virus spread, which would cause multiple effects in the most vulnerable population who would suffer an infection by 2019- nCoV.

Morpholine phosphorodiamidate oligomers linked to peptide

Morpholine phosphorodiamidate oligomers (PPMO) are synthetic antisense molecules. These molecules target the genetic code within the virus. they avoid the virus reproduction ability since they can interfere with gene expression by sterically blocking complementary RNAs[14]. This synthetic compound interferes in the correct splicing in pre-mRNA of TMPRSS2 receptor, causing the mature mRNA production without the presence of exon 5. This leads to the incorrect genetic expression of the receptor. That is why these synthetic agents resemble single-stranded DNA, and can easily enter the target cell to change the pre-mRNA splicing pattern.

There are in vitro results that show us this molecule could contribute to the drastic reduction of SARS-CoV-2 in cell cultures. In others words, this molecule can reduce the contagion rate in a range of time of 16, 24, and 48 hours after the cell cultures were exposed to the virus[16].

Polyamide

Polyamide is a type of polymer that contains amide-type bonds. Many researches have used this polymer to develop different therapeutic treatment. Also, this polymer has been used to coat medical devices as it functions as an antimicrobial agent in the clinic devices[17].In this research, polyamide is suggested as another candidate drug to counteract respiratory infections caused by SARS-CoV-2 since its compounds moderately block the TMPRSS2 receptor; Therefore, this drug may moderately contribute to the non-adherence of some viruses that use this receptor as a route of entry to host cells, such as the current SARS-CoV-2[18-19].

Rubitecan, Lorazepam, Zinc15 and Ascorbic Acid

Rubitecan is an enantiomer 20 (S) enantiomer 9-nitrocamptothecin (9-NC). Its main function is to act as an inhibitor of tumor activity, presenting a blocking molecule that prevents cell replication. On the other hand, lorazepam is a drug from the benzodiazepine family, which is given as a treatment for short-term insomnia, hypnotics, and muscle spasms[20]. These target molecules have been tested in silico by docking and molecular dynamics. The test result showed a high binding affinity between these drugs (ligands) and the TMPRSS2 receptor (target molecule) Also, this test result could highlight a relatively low energy value that showed us that these molecules could be candidates to counteract the entry of the virus into the cell.

Likewise, the Zinc15 molecule has been studied as a potential inhibitor of the entry pathway through the receptor of the transmembrane protein TMPRSS2, which in molecular coupling has a very high affinity for this protein[21]. Also, Ascorbic acid or vitamin C can work as a weak antihistamine agent, which in turn contributes to the symptoms of lower respiratory infections. The inhibition of ACE II receptor in turn contributes to the non-expression of TMPRSS2, thus helping to mitigate flu symptoms and, in turn, vitamin C could be considered as another alternative to treat critically ill patients with covid19[22].

The aforementioned information highlights different candidate molecules that may be targets for inhibiting the spread of 2019-nCoV infection. The Table 1 shows us another summary about the characteristics of these drugs that could be successful to reduce SARS-CoV-2 infection.

CONCLUSION

All the drugs mentioned in this review could contribute to the aim of achieving the actions that prevent the entry of the SARS-CoV-2 virus by serine protease. Although the mechanisms of action and efficacy of these drugs require more rigorous studies to have full certain of their results in human beings, they cannot be ruled out as possible candidates, on the contrary, they must be taken into account as possible useful drugs in the treatment of COVID 19 patients.

Table 1. Characteristics of drugs that can be used to treatment 2019-ncov.

Drug	Mechanism of action	Metabolic pathways involved	Essays trials	Adverse effects
Camostat mesilate	TMPRSS2 receptor	block the activation of trypsinogen to trypsin and the path of the inflammatory cascade.	*In vitro* *In vivo*	Eosinophilic pneumonia, skin rash, itching, nausea and diarrhea.
Nafamostat	TMPRSS2 receptor	Inhibits the activities of proteases, esterase in the complement system, and factors in the coagulation system	*In vitro* *In vivo*	Agranulocytosis, hyperkalemia, anaphylaxis, dyspnea and cardiac arrest.
Bromhexine Chlorhydrate	TMPRSS2 receptor	Acts within the mucus-secreting cells and disrupts the structure of acid mucopolysaccharide fibres	*In vitro* *In vivo*	Headache, dizziness, nausea, rash, diarrhea, vomiting and indigestion.
PPMO	TMPRSS2 receptor	Block complementary RNAs of the pre-mRNA of the TMPRSS2 receptor	*In vitro*	No data available
Ascorbic Acid	ACE II and TMPRSS2 receptor	Works as an antioxidant against infections, collagen synthesis and detoxifying reactions	*In vitro*	Acute liver injury or jaundice.
Rubitecan	TMPRSS2 receptor	block via topoisomerase, DNA and RNA synthesis in dividing cells	*In silico* *In vitro*	Vomiting, cough, fatigue and pharyngeal mucositis
Lorazepam	TMPRSS2 receptor	Inhibits benzodiazepine receptors in the chloride channel	*In silico*	Agitation, delirium, insomnia, muscle Spasms, deep respiratory, depression, coma, and death
Zinc15	TMPRSS2 receptor	Inhibits the activities of proteases	*In silico* *In vitro*	Rash, itching, eye redness, ocular irritation and skin irritation

REFERENCES

1. Ruiz-Bravo A, Jimenez-Varela M. SARS-CoV-2 y pandemia de síndrome respiratorio agudo (COVID-19). Ars Pharm. 2020; *61*(2), 63-79. doi:10.30827/ars.v61i2.15177.
2. Montes A, Coronell W, Baldiris R. Can house flies mechanically carry and/or transport sars-cov-2? Int J Clin Virol. 2020; 4: 076-078. doi: 10.29328/journal.ijcv.1001019.
3. Corman V, Lienau J, Witzenrath M. Coronaviruses as the cause of respiratory infections. Der Internist. 2019; 60(11): 1136-1145. doi: 10.1007/s00108-019-00671-5.
4. WHO. Coronavirus diastase (COIVD-2019) situation reports [Internet] 2020 Available in: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/.
5. Liu J, Liao X, Qian S, Yuan J, Wang F, Liu Y, Zhang Z. Community Transmission of Severe Acute Respiratory Syndrome Coronavirus 2. Emerging infectious diseases Shenzhen, China. Emerg Infect Dis. 2020; 26(6). doi:10.3201/eid2606.200239.
6. Ong S, Tan Y, Chia P, Lee T, Ng O, Wong M, Marimuthu K. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA. 2020;323(16):1610–1612. doi:10.1001/jama.2020.322.7
7. Xiao K, Zhai J, Feng Y, Zhou N, Zhang X, Zou J, Zhang Z. Isolation and characterization of 2019-nCoV-like coronavirus from Malayan pangolins. BioRxiv. 2020; 02.17.951335; doi:10.1101/2020.02.17.951335.
8. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Müller M. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020; 181, 271–280. doi:10.1016/j.cell.2020.02.052
9. Ueda M, Uchimura K, Narita Y, Miyasato Y, Mizumoto T, Morinaga J, Shiraishi N. The serine protease inhibitor camostat mesilate attenuates the progression of chronic kidney disease through its antioxidant effects. Nephron 2015; 129:223-232. doi:10.1159/000375308.
10. Bittmann S, Luchter E, Weissenstein A, Villalon G, Moschüring-Alieva E. TMPRSS2-Inhibitors Play a Role in

Cell Entry Mechanism of COVID-19: An insight into Camostat and Nafamostat. J Regen Biol Med. 2020; 2(2): 1-3. doi:10.37191/Mapsci-2582-385X-2(2)-022

11. Yamamoto M, Kiso M, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Imai M, Takeda M, Matsuda Z. The anticoagulant nafamostat potently inhibits SARS-CoV-2 infection in vitro: an existing drug with multiple possible therapeutic effects. bioRxiv. 2020.04.22.054981; doi:10.1101/2020.04.22.054981
12. Lucas J, Heinlein C, Kim T, Hernandez S, Malik M, True L, Clegg N. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. Cancer Discov. 2014; 4(11): 1310-1325. doi: 10.1158/2159-8290. CD-13-1010.
13. Shen L, Mao H, Wu Y, Tanaka Y, Zhang W. TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie*. 2017;142:1-10. doi:10.1016/j.biochi.2017.07.016
14. Böttcher-Friebertshäuser E, Stein D, Klenk H, Garten W. Inhibition of influenza virus infection in human airway cell cultures by an antisense peptide-conjugated morpholino oligomer targeting the hemagglutinin-activating protease TMPRSS2. J Virol. 2011; 85(4): 1554-1562. doi: 10.1128/JVI.01294-10
15. Limburg H, Harbig A, Bestle D, Stein D, Moulton H, Jaeger J, Koczulla A. TMPRSS2 is the major activating protease of influenza A virus in primary human airway cells and influenza B virus in human type II pneumocytes. J Virol. 2019; 93(21): e00649-19. doi:10.1128/JVI.00649-19.
16. Bestle D, Heindl M, Limburg H, Pilgram O, Moulton H, Stein D, Becker S. TMPRSS2 and furin are both essential for proteolytic activation and spread of SARS-CoV-2 in human airway epithelial cells and provide promising drug targets. bioRxiv. 2020.04.15.042085; doi:10.1101/2020.04.15.042085
17. Zuñiga J, Cortes A. The role of additive manufacturing and antimicrobial polymers in the COVID-19 pandemic. Expert Rev Med Devices. 2020.17:6, 477-481, doi: 10.1080/17434440.2020.1756771
18. Nickols N, Dervan P. Suppression of androgen receptor-mediated gene expression by a sequence-specific DNA-binding polyamide. PNAS. 2007; 104(25): 10418-10423. doi: 10.1073/pnas.0704217104.
19. Puc J, Kozbial P, Li W, Tan Y, Liu Z, Suter T, Ohgi K, Zhang J, Aggarwal A. Rosenfeld M.G. Ligand-dependent enhancer activation regulated by topoisomerase-I activity. Cell. 2015; 160:367–380.doi: 10.1016/j.cell.2014.12.023
20. Corponi F, Fabbri C, Bitter I, Montgomery S, Vieta E, Kasper S, Pallanti S, Serretti A. Novel antipsychotics specificity profile: A clinically oriented review of lurasidone, brexpiprazole, cariprazine and lumateperone. Eur Neuropsypharm. 2019; 29(9): 971–985. doi:10.1016/j.euroneuro.2019.06.008
21. Elmezayen A, Al-Obaidi A, Şahin A. T, Yelekçi K. Drug repurposing for coronavirus (COVID-19): in silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. J Biomol Struct Dyn 2020; 1-12.doi: 10.1080/07391102.2020.1758791
22. Alan C, Real J. P, Palma S. D. High Dose Of Ascorbic Acid Used In Sars Covid-19 Treatment: Scientific And Clinical Support For Its Therapeutic Implementation. Ars Pharm. 2020. 61(2), 145-148.doi: 10.30827/ars.v61i2.15164.