Protease Inhibitors: Strategy to Disrupt COVID 19 Contagion

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Abstract

Background:

The COVID 2019 (Corona Virus Disease) has been currently going pandemic as per WHO (World Health Organisation) situation reports. The major differentiating point in this virus is the presence of a unique furin cleavage site. Our insilico study points out to the effectiveness of a potent plant origin furin inhibitor. We exploited the aspect of the cleavage machinery of furin which is critical and indispensable for the entry of SARS-CoV-2 (Severe Acute Respiratory Syndrome – Corona Virus 2) in human cells and subsequent massive contagion. Most of the drugs are presently targeting ACEII (Angiotensin Converting Enzyme 2) receptor. But these options have injurious effects in diabetics and cardiac patients. Repurposing of old drugs are the current undergoing therapeutic measures as per the EUA (Emergency Usage Administration) of Food and Drug Administration. These aspects could be avoided if we develop a better and safer strategized drug .

Methods

In silico analysis of plant based protein analogs to inhibit furin protein.

Results

Significant interactions of proposed analogs of protease inhibitor of plant origin against furin protein. Furin inhibitors would be incredible drug targets against cancers, MERS CoV (Middle East Respiratory Syndrome) and most urgently SARS- CoV-2.

Conclusion

We report the first of its kind of furin inhibitor and believe that this major finding would fast track arenas for drug trials.

Background:

The COVID 2019 outbreak gained a status of “Public Health Emergency of International Concern” on 30th January, 2020.¹ The novel corona virus was found in unknown causes of pneumonia in China in late 2019 and on 11th February, 2020 the World Health Organisation (WHO) named it as Corona virus disease (COVID-19).² Eventually, due to the aggressive and massive increase in the number of cases (118,000) spreading across 110 countries and territories around the world, the WHO declared it as a pandemic on 11th March, 2020. The present status of the pandemic can be tracked in real time through the following website (<https://www.who.int/emergencies.arcgis.com/experience>).³ Till date, there are 6,38,146

confirmed cases and 30,105 deaths globally (Data as reported by national authorities by 16:00 CET, 29th March, 2020). This has triggered a lot of research trials related to vaccine development and possible therapeutic measures to combat the disease.

It was in the year 1966 that coronaviruses were morphologically described as spherical having a core shell and surface projections all over.⁴ The term “corona” in Latin means “crown”. They are categorised into alpha, beta gamma and delta subfamilies. Of these, alpha and beta coronaviruses are of mammalian origin (bats) while gamma and delta have pig and bird origins respectively. SARS CoV-2 belongs to beta-coronaviruses and is a novel relative of SARS CoV classic virus.^{5,6} It is also reported to have 96% similarity to bat coronavirus genome.⁶ The main structural genes of this novel corona virus are N (nucleocapsid protein), S (spike protein), SM (a small membrane protein), and the (M) membrane glycoprotein.⁷ Zhu et al and Wu et al, 2020, published the sequence of the viral genome.^{8,9}

There has been pandemic episodes in 2003 and 2011 from two other coronaviruses namely (Severe Acute Respiratory syndrome) SARS and MERS (Middle East Respiratory Syndrome) originating in China and Saudi Arabia respectively.^{10,11} SARS-CoV-2 is the seventh discovered coronaviruses in humans.⁸

The pathogenicity of any virus would largely depend on its mode of entry and sustainability in the host cell. Racaniello, 2020 has elaborated in his virology blog about the novelty in the spike glycoprotein of the virus SARS-CoV-2. He explained the major differences in the behaviour of spike glycoprotein (S) in the SARS-CoV-2 and SARS classic. The furin proteases can potentially cleave the spike protein of the novel coronavirus due the presence of a cleavage site (PRRARS|V). This character varies among the coronaviruses and is also a vital factor for the virus to jump to other species.¹² The most similar bat coronavirus based on sequence homology does not have this cleavage site. It has been being assumed that this “gain of function” in SARS classic helped it to jump into humans from the wildlife market in China.

The pandemic nature of SARS-CoV-2 is due to its ability to bind to ever present furin in many tissues of humans.

Much of the research focus is on the mechanism of cleavage of the spike protein of SARS-CoV-2, its fusion with ACE2 (human angiotensin-converting enzyme 2) receptor in human cells and its entry. Hypothetically, this cleavage is mediated by furin protease. In SARS classic the cleavage occurs with much more difficulty.¹³ than SARS-CoV-2 whereas SARS classic also uses the ACE2 receptor for its entry.¹⁴ The cellular receptor of MERS coronavirus is dipeptidyl peptidase 4 (DPP4).¹⁵

In the present study, we propose a furin inhibitor through in silico approaches from plant origin which could be a promising therapeutic aspect to disrupt the cleavage of S glycoprotein and hence block its entry into the human cell.

Results:

Table 3: Computational analysis showing receptor ligand interaction

RECEPTOR AND LIGAND	E. TOTAL	RECEPTOR AND LIGAND	E. TOTAL	RECEPTOR AND LIGAND	E. TOTAL	RECEPTOR AND LIGAND	E. TOTAL
FURIN And ANALOG I	-544.5	FURIN And SGP I	-725.4	SGP I with Furin And ANALOG I	-684.3	SGP II with Furin And ANALOG I	-636.7
FURIN AndANALOG II	-638.5	FURIN And SGP II	-704.7	SGP I with Furin And ANALOG II	-701.8	SGP II with Furin And ANALOG II	-626.0
FURIN AndANALOG III	-741.6			SGP I with Furin And ANALOG III	-632.4	SGP II with Furin And ANALOG III	-765.4

(Note: SGP – Spike Glycoprotein I And II)

We studied three natural analog interaction of plant origin against furin protease. Analog III (miraucilin like protein) was the most potent inhibitor against furin protease (E Value – 741.6). Furin protease was interacting better with spike glycoprotein I (2019-nCoV S2 subunit2) than spike glycoprotein II (2019-nCoV chimeric RBD) (E Value – 725.4). The spike glycoprotein I along with furin protease was studied for interaction with analog I, II and III. Of these, analog II (trypsin inhibitor) interacted best of all the inhibitors (E Value – 701.8). Again, the spike glycoprotein II along with furin protease was studied for interaction with the 3 analogs of which, analog III (miraucilin like protein) was found to be the best inhibitor (E Value – 765.4) (Table 3).

Methodology:

The following computational approaches and bioinformatics tools have been used throughout the study.

Table 1
Computational approaches and tools used:

Bioinformatics tools	Description	Uses
BLAST	Convert the nucleotide sequence to protein sequence	To get protein sequence from nucleotide sequence.
Swiss Model	Prediction and create 3d-structure of protein	To create the 3d structure of Spike glycoprotein of COVID-19, Furin protease, and natural protease inhibitor
Hex 6.12	Molecular Docking	To perform receptor ligand docking
CAST p 3.0	For identifying and measuring the pockets.	To measure the effectiveness of receptor and ligand interaction.

Table 2
Experimental Protocol

Set	EXPERIMENT	Link
1	Modelling the Furin protease of by Swiss-model	https://swissmodel.expasy.org/assess/3RJWkp/01
	Modelling the Spike glycoprotein of COVID 19 by Swiss-model	https://swissmodel.expasy.org/assess/V64Zdn/01 https://swissmodel.expasy.org/assess/V64Zdn/02
	Modelling the Natural protease inhibitor by Swiss-model	https://swissmodel.expasy.org/assess/M2JWtk/03 https://swissmodel.expasy.org/assess/M2JWtk/04 https://swissmodel.expasy.org/assess/M2JWtk/02
2	Docking in between furin protease of human with Natural protease inhibitor (analog) by the help of Hex6.12.	N/A
3	Docking between Furin protease and different Spike Glycoprotein by the help of HEX 6.	N/A
4	Docking between Spike Glycoprotein I with Furin Protease and different Protease Inhibitor (Analog) by the help of HEX 6.12	N/A
5	Docking between Spike Glycoprotein II with Furin Protease and different Protease Inhibitor (Analog) by the help of HEX 6.12	N/A
6	Analyze the docking result by CASTp 3.0.	http://sts.bioe.uic.edu/castp/index.html?j_5e7c8c2893ed5

Discussion:

The current study has focussed to find out a natural protease inhibitor to block the action of furin. In SARS-CoV-2 the furin enzyme plays a key role to cleave the spike glycoprotein which is the first step to initiate an infection. It is noteworthy to mention that the S glycoprotein is an attractive target for vaccination trials as it projects out on the surface of SARS-CoV-2.¹⁶ Studies have also mentioned that SARS-CoV-2 has a furin cleavage site at the junction of subunits S1 and S2.^{17,18,19} The spike protein is made up of two subunits S1 and S2 which have distinct functions. The S1 subunit has the receptor binding machinery and binds to ACE2 receptor in the human cells and helps to stabilize the prefusion stage of the membrane connected to S2 subunit which has the fusion machinery between the viral and

cellular membrane.¹⁶ This gives us a clear insight that furin could also be a very important drug or a vaccine target to disrupt the binding and fusion machinery required by coronaviruses.

A correspondence was published saying that the coronaviruses do not survive long on surfaces such as steel, plastic etc but need potential hosts to sustain.²⁰ This fusion mechanism of SARS-CoV-2 is common to MERS also.¹⁷ Other studies have targeted the ACE2 receptor.^{21,22} Xia et al, 2020 have designed furin inhibitors for SARS CoV as well as MERS CoV.¹⁴ However, still a very critical point lies in using ACE2 inhibitors as vaccine or drug targets. It was observed that ACE2 receptor targets are those which have been used in treatments for diabetics, heart disease etc.^{23,24} Additionally, ACE2 receptors are present in intestine, kidney, oral mucosa and heart endothelium.^{21,24} Eventually, overexpression of ACE receptors result due to administration of ACE inhibitors as reported in rat model experiments.^{25,26} As the entry of SARS-Co-2 is mediated by ACE 2 receptor, people undergoing drug therapies for diabetics, hypertension etc would be more susceptible to SARS-CoV-2 as they would be inherently overexpressing ACE2 receptors.²³ This might be the most valid reason why diabetic and heart patients are at larger risk for SARS-Cov-2.²⁷ Thus, it is the most urgent need to develop a safer and effective therapy. It is noteworthy to mention that besides being anticipated targets for SARS- CoV-2, furin inhibitors have had excellent results in cancer therapy. This matter is beyond the scope of this paper but it clearly exhibits the multipotent therapeutic aspect of a furin inhibitor. Furin was found to overexpress in different cancer cells and its role in cancer related proprotein proteases.²⁸ Contour et al has enlisted a summary of patent applications in furin inhibitors.²⁹ Millet and Whittaker 2014, observed that high levels of MERS CoV infection had a correlation with overexpression with furin.¹⁷ They mentioned that MERS CoV entry could be blocked through siRNA silencing.

Conclusion:

Thus it is worth believing that furin inhibitors would be incredible drug targets against cancers, MERS Co V and most urgently SARS-CoV-2. Our study is limited to insilico approaches. Henceforth it is anticipated that purified plant protein of analogs that we reported would fast track validation of our findings.

Abbreviations

CoVID

Corona Virus Disease

WHO

World Health Organisation

SARS CoV 2

Severe Acute Respiratory Syndrome Corona Virus 2

EUA

Emergency Usage Administration

DPP4

Declarations

Ethics Approval and consent to participate:

NA

Availability of data and material:

Yes, data sharing is possible

Competing Interest:

None

Funding:

NA

Authors contribution:

Dhiman Chandra Paul performed and interpreted the in silico analysis; Dr Minakshi Bhattacharjee originated the research idea and wrote the paper; Dr Manash Pratim Sarma gave critical insights in virology aspects of the study. (Both 1st and 2nd author have equal contribution to the research work).

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Figures

Figure 1

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