International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Analyzing the Effect of Natural Protease Inhibitor (BBI) on Replication of COVID-19 in Comparison to HIV, using Protein Interaction Studies

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Abstract: ***COVID-19 is the disease caused by coronavirus, known as SARS2, which has emerged into a pandemic. Many approaches are being made for designing a drug against it. In this study we have studied the replication of COVID-19 and its structural and functional similarities with HIV. The studies conducted before had suggested that the anti-retroviral drugs used in HIV has good affinity towards COVID-19 and hence help in curing it, example: Lopinavir, ritonavir, etc. Similarly in this study we have come across the natural protease inhibitors namely BBI (Bowman Birk Inhibitor) a serine protease inhibitor, from soybean that has inhibitory effects on HIV. The structural similarities between the two suggest that this can also be effective in treating COVID-19. To support this hypothesis the binding affinity of this inhibitor is checked using Hex 8.0.0 and the results are interpretated accordingly. Thus the structural aspects of it can be used as drug targets to treat it.***

Keywords: COVID-19, HIV, Protease inhibitors, BBI, Bowman Birk Inhibitor, Protein-protein interaction, Anti-retroviral drugs

1. Introduction

1.1 Covid-19 general info

Coronaviruses belong to the Coronavirinae subfamily of the family Coronaviridae and order Nidovirales[2][1]. In year 2019 the disease outbreak, which is now a pandemic that originated in china is identified as coronavirus. This is now known as SARS2 (Severe Acute Respiratory Syndrome 2), which causes the disease what we call COVID-19. The SARS CoV2 belongs to β genus.

1.2 COVID-19 structure

The diameter of each virion is 50-200nm. SARS CoV2 has four structural proteins, namely spike protein(S), Membrane(M), Envelop(E) and Nucleocapsid(N).[3][4] The S, E, M proteins together form the viral envelop whereas the N protein helps form the viral genome during replication. Amongst this the protein responsible for attachment and membrane fusion is the spike(S) protein, which is made up of two subunits namely S1 responsible for attachment of virion and S2for membrane fusion. The genome of SARS CoV2 is positive stranded RNA molecule.

1.3 Significance of spike protein in COVID

The spike protein is a transmembrane protein, which is heavily glycosylated i.e. it has 21-35 N-glycosylation sites. This is assembles in a trimeric form on viral surface giving it a crown like(corona) appearance.[5][6] The ectodomain in the two domains share the same organization: S1 N-terminal which helps in receptor binding and S2 C-terminal responsible for membrane fusion and contains the putative fusion peptide and heptad repeat H1 and H2.This homotrimeric spike glycoprotein help in binding to the respective cellular receptors which leads to cascade of events that allow the fusion of membranes and thereby facilitate cell entry. The scientific evidence shows that the receptor of SARS and SARS CoV2 is same i.e. the ACE2 (Angiotensin converting enzyme 2) receptor.

2. Methodology

2.1Pathogenesis of COVID-19

2.1.1Mechanism of human intervention-

The spike, which is made up of two subunits namely S1 and S2, binds to the ACE2 receptor according to the structural and functional analysis[7]. These are expressed mostly in liver, ileum, kidney, heart and bladder, amongst which it is abundantly found in lung epithelial cells. The spike protein undergo protease cleavage after binding to the host protein, this is a two-step event. First is the cleaving of S1/S2 cleavage site for priming and second is S'2 site activation by cleavage, which is the adjacent position to fusion peptide within S2 subunit.Once the SARS CoV2 attaches to target cell,the protease TMPRSS2 on cell opens the spike protein and the fusion peptide in the S2 subunit and host receptor ACE2 is exposed. This leads to fusion of membranes and the virion enters the cell by forming of endosome around the virion. The cleavage at S1/S2 site, enables the S1 and S2 non-covalently binding and stabilization of membrane anchored S2 subunit at prefusion site by distal S1 subunit. The activation of spike membrane fusion via irreversible conformational changes is done by subsequent cleavage at S'2 site. The uniqueness provided to the SARS CoV2 is by the furin cleavage site('RPPA" sequence) present at S1/S2 site. The pathogenicity of virus is increased due to the expression of this furin.

2.1.2 Mechanism of infection/replication (normal/ spike protein)

Attachment and Entry: The interaction between S protein and its receptor on host cell facilitates initial attachment of virion to it.[8] After receptor binding the virion has to enter the host cell cytosol. For entry of virion the S protein is proteolytically cleaved by cathepsin, TMPRRS2 or another protease. This is then followed by fusion of the membranes

of cell and virion. S protein is cleaved at the S2 subnuit at two sites, first cleavage leads to separating of RBD(Receptor Binding Domain) and fusion domains whereas the second cleavage exposes the fusion peptide. Acidified endosomes are the one where fusion generally takes place.

Replication and Transcription: The genomic RNA of coronavirus encodes for structural, that plays a critical role in RNA synthesis, as well as non-structural proteins that appear to have selective advantage in vivo and not replication. The translation of genomic RNA mediates the expression of replicase-transcriptase protein genes. [10]Translation of replicase gene is the gene that is responsible for expression of co-terminal polyproteins, namely pp1a and pp1ab, which are encoded by two large ORFs,rep1a and rep1b. These polyproteins contain nsps 1-11 and 1-16, respectively. The nsp11 in pp1a becomes nsp12 in pp1ab polyprotein. These polyproteins are cleaved to yield subsequent number of nsps. This is done by the two or three proteases, encoded by coronavirus, capable of cleaving the replicase polyproteins, namely papin-like proteases(PLpro) and serine type protease, the main protease(Mpro). Thus the generated nsps and cellular proteins now assemble to from a replicase-transcriptase complex(RTC). The complexes thus formed accumulates as perinuclear regions and get associated as double membrane vesicles. The nascent pp1a/pp1ab polyproteins get anchored to the membrane during first step of RTC formation which is done by hydrophobic transmembrane domains present in nsp3, nsp4 and nsp6. The vesicles containing virions then fuse with the plasma membrane to finally release the virus, which then attaches to the new cell and repeats the cycle. [9][8]

Assembly and Release: Following the replication of sub-genomic RNA, the structural proteins are translated (S, E, N, M) and are then inserted into ER (endoplasmic reticulum)[10]. These then enter the ERGIC (endoplasmic reticulum- Golgi intermediate compartment). In this the viral N protein, encapsulated, from mature virions by budding into the membrane of ERGIC containing structural proteins. The mature virions are then transported to the membrane in vesicles, which then are finally released by exocytosis. The pathway of transport of virion vesicle from golgi to membrane is not yet clear. The S protein that doesn't get assembled with virion form a giant multinucleated cells, in some coronaviruses, by transisting to cell surface and mediating the cell-cell fusion of infected and uninfected cell. This doesn't get detected by viral specific antibodies as well and spreads within the body.

2.2 Relation between HIV and COVID-19

2.2.1Structural similarity:

The spikes, upon the envelop, is the common feature of COVID-19 and HIV[11]. These proteins are initially synthesized as precursors and then processed as transmembrane subunits i.e. gp41 in HIV and S2 in COVID-19, that lead to membrane fusion, and a surface subunit that helps in interaction with cell receptors. This proteins contains the hepad repeats at the C and N-terminus of both the fusion proteins and the cysteine residues between the two giving it a loop structure also the aromatic residues rich motifs with the transmembrane segment at end.

2.2.2 Functional similarities of the proteases

The two main proteases belonging to them have similar functions, the HIV-1 protease and 3-chymptrypsin like protease in HIV and COVID-19 respectively. The entry of HIV-RNA is accompanied by the reverse transcriptase (converts viral RNA to DNA), integrase (incorporates viral genetic information into host cell DNA)and mature HIV-1 protease. This is then transcribed and translated by host cell in Gag-Pol polyprotein, which is then cleaved into functional proteins by HIV-1 protease. Similarly the polyproteins produced by COVID-19 are cleaved into respective proteins by PLpro (papin like protease) and 3CLpro(3 chymotrypsin like protease). Hence the 3-chymotrypsin like cysteine protease enzyme is essential for its life cycle.

2.2.3: HIV retrovirals in COVID-19

The use of anti-retroviral drugs of HIV have been approved for the treatment of COVID-19 as the drugs showed a positive impact. [16] The efficiency of ARV's against three diseases caused by coronaviruses: COVID, SARS and MERS was summarized in the recent review. [17] Amongst this the most studied ARV is **ritonavir boosted lopinavir** that is a HIV protease inhibitor. This is found to be effective on COVID-19 as well. The mode of action is same as in HIV, it inhibits the main protease in COVID-19 and thus stop its replication.

2.3 Drugs Used In Treatment of COVID:

COVID DRUGS:[12]
Remdesivir, Chloroquine and hydroxychloroquine, Lopinavir and ritonavir, Nafamostat and camostat, Famotidine, Umifenovir, Nitazoxanide, Ivermectin, Corticosteroids, Tocilizumab and sarilumab, Bevacizumab, Fluvoxamine.

2.4 Can human intake help on curtailing the replication of COVID-19?

2.4.1Possible Intake:

The food that can help in curtailing the replication of covid-19 are-
Great variety of plants including legumes and cereals and certain fruits(apples, bananas, pineapples and raisins)and vegetables(spinach, cabbage, cucumbers, potatoes and tomatoes)

2.4.2 Significance of it:

The above mentioned food contains trypsin, chymotrypsin, papain and cysteine protease inhibitors.

2.4.3 How does it help?

There are some protease inhibitors in plants that can inhibit mammalian plasma serine proteases namely kallikerin and plasmin, and plant sulfhydryl proteases namely bromelian, ficin and papain. Amongst them chymotrypsin protease inhibitor is the one in which we are interested, which is abundantly found in soybean. Protease inhibitors, as we know, are present in all legumes but the trypsin inhibitor in

soybean is particularly extensively examined as soy protein is the very important component in animal and human nutrition. As we studied earlier, COVID-19 requires 3CLpro for cleaving of the polyprotein into functional viral proteins, which can be inhibited by a protease inhibitor trypsin-chymotrypsin present in soybean.

2.5 Comparative analysis of interaction of protease inhibitor in SARS and HIV:

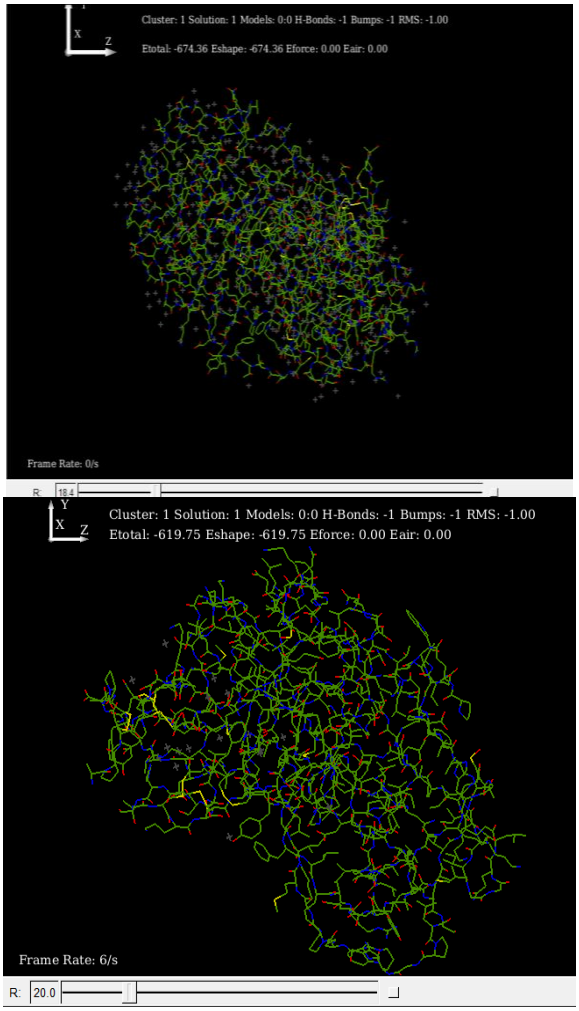

Figure 1: Protein-protein (SARS CoV2 and BBI)Fig. 2: protein-protein (HIV-1 pro and BBI) docking using hex 8.0.0 docking from hex 8.0.0

The binding affinity of the BBI (Bowman Birk inhibitor) protease inhibitor from soybean with that of 3CLpro protease of SARS CoV2 and the binding affinity of the BBI and HIV-1 protease was checked, using the Hex 8.0.0 software.

Table 1: This table provides us with the result of the above protein interactions

Receptor	*Ligand*	RMS Value	Etotal
SARS CoV2 3CLpro protease (3D structure)	Bowman Birk inhibitor (from soybean)	-1	-674.36
HIV HIV-1 protease (3D structure)	Bowman Birk inhibitor (from soybean)	-1	-619.75

The more negative the Etotal value more strong the interaction between the proteins i.e. from the above table we can interpret that the BBI from soybean has more affinity (binds strongly) towards COVID-19 than HIV.

2.6 Can it be a potential inhibitor for covid-19 replication?

The main proteases in HIV and COVID-19 have similar functions i.e. replication, currently antiviral drug that inhibits the replication of HIV was found to be affective on COVID-19 protease as well and thereby inhibit its replication. [13]

The study conducted by the researchers earlier demonstrates that BBI, a natural product from soy foods, has ability to potentially inhibit HIV infection[14]. The inhibitory effect was found to be highly effective i.e. about >90% inhibition, at a dose of 25µg/ml or higher. Complete inhibition of HIV infection was observed when treated with BBI, this was also significant before or after infection in macrophages. Through this comparative study we can conclude that, as the BBI can inhibit the replication of HIV without cytotoxicity so it can also inhibit the replication of COVID-19, similar to the approved anti-retroviral drugs of HIV.

BBI is resistant to pH range and proteolytic enzymes in gastrointestinal tract (GIT), bioavailable and non-allergic, [18] hence is a unique protein. This resistance of BBI to extreme conditions favors its transport across the gut epithelium which allows it to distribute to target organs and exert its beneficial affects there. [17] Hence it can act as a potential natural inhibitor with very negligible side effects.

3. Conclusion

In the above work the structural components, the viral proteins and their significance and the complete genomic replication of COVID -19 was studied. Further the comparative study of HIV and COVID-19 was done, which includes the structural similarities between the two along with the functional similarities between them. The drugs used for treatment in COVID were enlisted and the also the natural products that can help in curtailing the replication were studied, amongst them soybean was found to be the significant one as it contains BBI protease inhibitor (chymotrypsin inhibitor) in abundance. The comparative analysis of binding affinity of BBI with HIV and SARS CoV2 was done using hex 8.0.0 software which proved that this inhibitor has greater affinity towards main protease of SARS CoV2 than HIV and as BBI has the ability to inhibit replication of HIV it can also be a potential inhibitor for COVID-19 replication. Investigation can be done by in vivo approach further.

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