

Review Article

Proposed drug interventions for SARS CoV 2 infection

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ABSTRACT

As the pandemic has already taken lots of lives across the globe, there's an urgent need for finding treatment options that might help in protection of infected people by either slowing or preventing the progression of the disease. It's important to understand the structure of virus, the mechanism by which it enters the host cell, replicates and infects other cells causing progression of the disease. This article focuses on use of already available and approved drugs for treatment of corona virus based on their mechanism of action and the structure and the life cycle of the virus.

Keywords: Corona virus treatment, Covid 19 drug intervention, Covid 19 treatment

INTRODUCTION

As the pandemic has already taken lots of lives across the globe, there's an urgent need for finding treatment options that might help in protection of infected people by either slowing or preventing the progression of the disease. It's important to understand the structure of virus, the mechanism by which it enters the host cell, replicates and infects other cells causing progression of the disease.

It has been noticed that the SARS CoV 2 virus has 82% nucleotide identity to the SARS CoV 1 virus and 89% to the bat SARS-like-CoVZXC21.¹

STRUCTURE

It is an encapsulated virus with non segmented positive sense single strand RNA.

Similar to the SARS CoV 1 virus it appears to be a part of beta group of sub family coronaviridae belonging to coronavirinae family of viruses which belongs to the Nidovirales order.²

Structurally corona viruses contains S protein, M proteins, E protein and N protein. S protein or the spike protein (s1 and s2), is a trimeric Class 1 fusion protein plays a role in attachment of virus to host cell, uses its N terminal for the same.^{3,4} M protein the membrane protein, it is the most abundant protein, has a large C terminal and a small N terminal and is responsible for providing shape to the virus.^{5,6} E protein is thought to be a transmembranous protein responsible for release of new formed viruses, in SARS CoV it is supposed to play an important role in pathogenicity.^{7,8}

N protein is present in Nucleocapsid and has two separate domains, the N terminal and C terminal domain (NTD and CTD), both of these bind to RNA and are important in tethering the RNA to the RTC (replication-transcription complex) and for packaging the genome into the new capsids.⁹⁻¹²

Apart from the mentioned ones, another protein called the HE (hemagglutinin esterase) might also be present in some, it binds to sialic acid on host cell surface glycoproteins and can show acetyl esterase activity, may help S protein in attaching to the cell.¹³

GENETIC STRUCTURE AND GENOMIC REPLICATION

The RNA has 5' cap structure and 3' poly A tail that enables it to act as messenger RNA and it can be used to translate proteins. It contains sequences which code for nsp (non-structural proteins) which are located in the replicase gene and occupies the majority of the genome. It also has some regions which remain untranslated called as UTR (untranslated regions), and these are located on both 5' and 3' ends. TRS (transcriptional regulatory sequences) are present in the initial part of all structural and accessory genes are important for the expression of the same.

RNA can directly translate polyprotein 1a and 1b (pp1a/pp1ab), which encodes nsps forming RTC (replication-transcription complex) in vesicles produced by ERGIC (endoplasmic reticulum golgi intermediate compartment).¹

IMPORTANT PHASIS IN VIRAL CYCLE

1. Attachment and entry into the cells
2. Replicase protein expression
3. Genome replication and transcription
4. Assembly of constituent proteins and genome to form virus
5. Release of viruses to ECF or directly to other cells

Authors will now focus only on important aspects where drugs might act and help in preventing spread or progression of disease in all of the above mentioned phases (Figure 1).

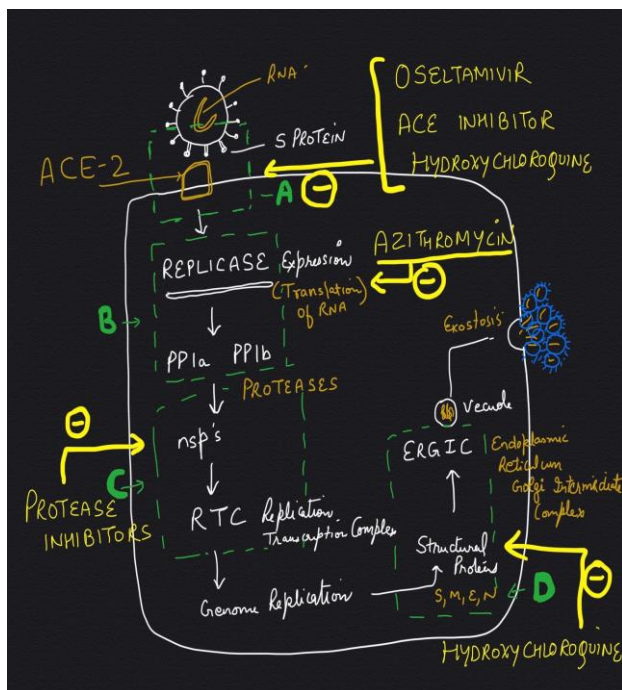


Figure 1: Life cycle of virus and possible drug interactions.

Attachment and entry into the cells

Corona viruses attachment starts with interaction of the S protein with the surface receptors, the receptor binding domains (RBD) are located on S1 and fusion sequence on S2. There are different receptors like DPP4, ACE2, CEACAM1, APN which are used by different corona viruses. SARS CoV 2 uses ACE 2 receptor. However, entry can occur in absence of the enzymatic domain of these receptors.^{3,14-16}

Proposed intervention point A

For attaching to cells, HE protein also helps, it binds to sialic acid on host cell surface, which is a part of glycoproteins. Sialic acid for being available for HE needs to be cleaved from glycoproteins which is carried out by neuroaminidases.

Proposed intervention point B

After binding the virus needs to access the cytoplasm of the host cell. The S protein is cleaved twice, first cleavage is done between S1 and S2, separating both and is carried out by proteases like CATHEPSIN leading to fusion of the membranes. Second occurs within S2, exposing the fusion peptide. This fusion peptide inserts into the membrane followed by joining of 2 heptad repeats forming a anti parallel 6 helical bundle/bridge through which the cytosol, its contents and the viral genome enters the host cell.^{3,14}

Important

The first cleavage is carried by acid dependant protease - cathepsin, the second cleavage of S2 occurs within Acidified lysosomes.

Proposed intervention point C

Replicase protein expression

The RNA genome translates to produce pp1a and pp1b by using frame shift mechanism aided by pseudoknot bocks. Once translation is over, the resultant polyprotein needs to be cleaved into its constituents. It is done by proteases like P1pro and Mpro. The cleavage and production of individual nsps are responsible for decrease and altered innate immune response.¹⁷⁻²⁰ The P1pro can alter and decrease Ub-dependent cellular responses to viral infection.²¹

Proposed intervention point D

Genome replication and transcription

RNA replication starts following the translation, it produces both genomic and sub genomic (negative sense RNA) fragments. The RNA polymerase recognises leader sequences of TRS (TRS L) and starts replication however

it stops at at particular TRS B (body), and starts synthesising the sub genomic negative sense RNA fragments as well.^{22,23} The N protein tightly binds the RNA in a bead on string pattern, two specific substrates have been identified on RNA for N protein, these are TRSs and genomic packaging signal.^{24,25}

Proposed intervention point E

Assembly of constituent proteins and genome to form virus

After replication of genome is completed, structural proteins are translated, S, E, M and are incorporated into the Endoplasmic reitculum (ER) from where they pass along the ERGIC (endoplasmic reticulum golgi intermediate compartment).^{26,27} N protein which is covering the genome is bud into the membrane of vesicles containing structural proteins.²⁸ M and E proteins function together to form envelope, E protein also plays a role in release of viruses by altering host secretory pathways.^{29,30} (Figure 5).

Release of viruses to ECF or directly to other cells

Once the virions are ready they are transported within the vesicles to the cell membrane and by exostosis it allows the newly formed viruses to spread to extra cellular space, where it can be detected and can also infect other cells.

Important

The glycosylation in golgi apparatus and transport requires acidic environment and be altered due to presence of basic substances.³¹

Proposed intervention point F

However in some cases the S protein can directly attach to adjacent cell forming a cytoplasmic connection which can be used for direct transfer of encapsulated virion to neighbouring cells without being spilled in extra cellular space and without being detected.

Proposed intervention point G

Possible drug interventions

Already few drugs have been used in various parts of the world with uncertainty, here try to provide list of drugs and their mechanism by which they may prevent the progression of disease.

Oseltamavir

It is a viral neuraminidase inhibitor, used in viral infections where viruses use neuroaminidase to bind to glycoproteins of host cell membrane for splitting sailic

acid and releasing the new viral progeny into extracellular space.

In corona virus, the main effect could be at the binding site. Oseltamavir may prevent formation of sailic acid on surface which is required by HE protein for helping S protien in attaching to host cell.

At intervention point B

Hydroxychloroquine / chloroquine

It is a 4-Aminoquinoline, used as anti malarial drug. It gets accumulated in lysosomal vecuoles of parasite causing altered heme polymerisation leading to accumulation of toxic heme and death.

In corona virus - The affinity of drug to accumulate in higher concentration in LUNGS (100 folds), specifically lysosomes and endosomes can be used.

It is basic and can alter the pH of the endosomes where the splitting of fusion protein in S2 of corona virus takes place and can prevent further fusion of virus and host cell membranes.

At intervention point C

It can also be accumulated in vecuoles formed by golgi apparatus and alter the pH, golgi apparatus requires acidic pH for gylosylation and transport and hence the viral products once formed can be prevented form being assembled and transported to other cells (Figure 2) (Figure 4).

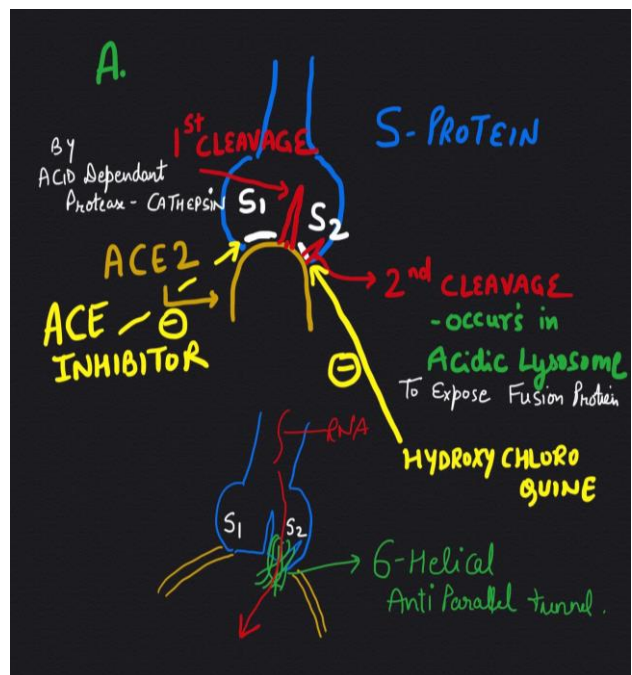


Figure 2: Attachment of S protein and cleavage of S1 and S2.

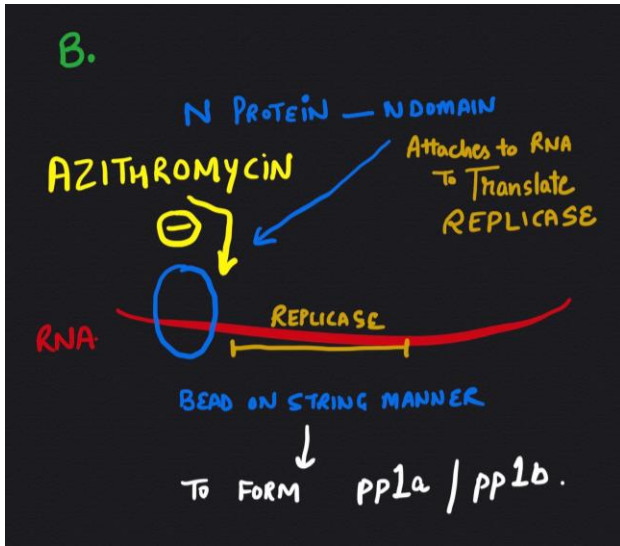


Figure 3: Expression of replicase gene.

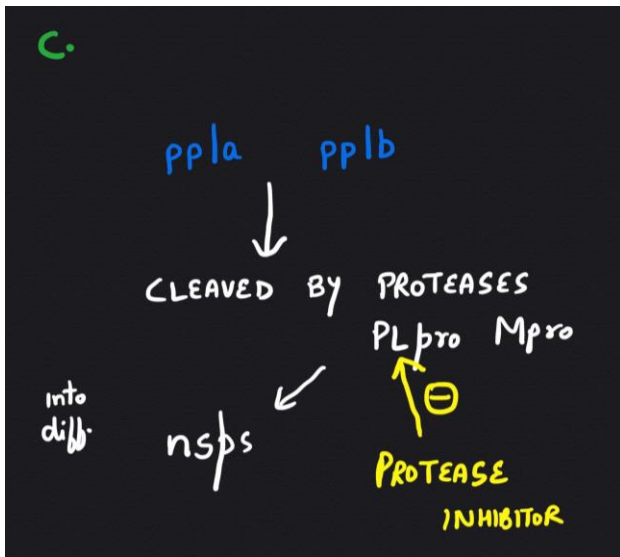


Figure 4: Splitting of Polyprotein 1a and 1b by protease.

At intervention point F

Protease inhibitor

Anti viral drugs used to treat HIV infections, they bind to the proteases that help in splitting of polyproteins in the vacuoles of ERGIC.^{32,33}

In corona virus - The similarity of HIV 1 aspartic proteases and Candida albicans Sap2 has already been demonstrated based on one or more substrate site similarity and hence the HIV protease inhibitors are known to act against other enzymes as well.³⁴

Response in patients with corona points the need for mapping the structural similarity between substrate sites

and exposed sites of Plpro protease and Mpro of corona virus and HIV 1 aspartic protease (Figure 3).

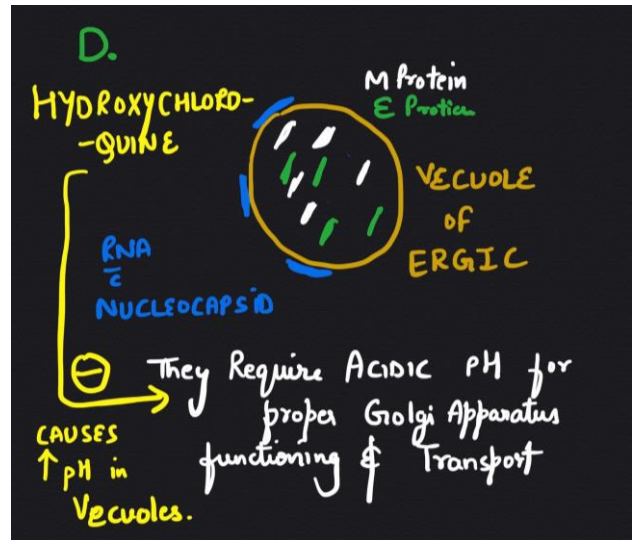


Figure 5: Assembly of virus proteins into virion inside ERGIC.

At intervention point D

Azithromycin (macrolides)

It is a macrolide that binds to 50s subunit of bacterial ribosome and interferes with translation. The macrolide binding site is composed primarily of RNA. Two segments of 23S rRNA, the central loop of domain V and the loop of hairpin 35 from domain II, are believed to be the major components of the drug binding site on the ribosome.³⁵ 50s sub unit also has highest number of positively charged basic residues of arginine and lysine which might play a role in binding of macrolides.³⁶

In corona virus - The binding of N protein to RNA via its N terminal has shown to be linked with high amount of Lysine and Arginine residues that binds to the RNA of the virus. Inside the N Terminal Domain of N protein of SARS-CoV, positively charged lysine and arginine residues have been proposed that bind a 32 nucleotide stem-loop structure located at the 3' end of the SARS-CoV RNA genome.^{37,38}

So Azithromycin can bind to the binding site rich in lysine and arginine on the N terminal domain of N protein of corona virus preventing it to bind to RNA and preventing the expression of replicase gene (Figure 2).

At intervention point D

ACE inhibitors

Bind to angiotensin converting enzymes in circulation as well as tissue proteins. Highly lipophilic variants like moexipril, ramipril show more affinity for tissue proteins.

In corona virus - They can work by 2 mechanisms, ace inhibitors will bind to ACE 2 receptors that reduces less number of ACE 2 proteins available for identification by virus.

The inhibition however will later lead to up regulation of ACE 2 expression, which will be helpful in patients in ARDS, it will prevent further progression of ARDS towards fibrosis by inhibiting of signaling pathways involved in tissue fibrosis and will also have additional benefits like cardioprotective and nephroprotective affect.^{39,40} (Figure 1).

At intervention point A

NaHCO₃ Nebulisation (in ARDS cases)

NaHCO₃ (sodium bicarbonate) - The airway acidifies in a variety of inflammatory lung diseases, airway alkalization improves absorption of cationic bronchodilators, such as albuterol and tiotropium, both in airway epithelia and smooth muscle cells.⁴¹

In corona virus - In cases of ARDS, pulmonary as well as lactic acid acidosis can occur, nebulisation even in absence of acidosis might be helpful by creating basic pH in the airway which hampers ACID dependant protease (cathepsin) to cleave S1 protein for attachment.

At intervention point A

Metabolisation of various drugs

1. Oseltamivir is activated in liver but excreted via urine.⁴²
2. Hydroxychloroquine is metabolised in liver by cytochrome P450 isoenzyme CYP2D6.⁴³
3. Lopinavir is metabolised exclusively by cytochrome P450 isoenzyme CYP3A and ritonavir inhibits the same and hence is used with lopinavir.⁴⁴
4. Ramipril is metabolised by liver and urine both to glucouronate conjugate in liver and diketopiperazine derivative in kidneys and mostly is excreted by urine.⁴⁵
5. Azithromycin is metabolised by liver but does not induce or affect any other medication.⁴⁶

A combination drug therapy based on multiple target sites might prove to be helpful in preventing further damage by suppressing the disease progression.

A combination of some of the drugs mentioned above might prove helpful severe patients whereas in mild cases drugs like azithromycin and hydroxychloroquine, either alone or in combination may prevent development of complications. Tab Azithromycin might also prove to be helpful as a prophylactic drug in patients with contact or infected patients without symptoms as it will prevent expression of replicase gene.

CONCLUSION

This article focuses on use of already available and approved drugs for treatment of corona virus based on their mechanism of action and the structure and the life cycle of the virus. Further research needs to be done for developing drugs with specific action against the novel corona virus, which could be targeted either to S protein receptor binding site, or against Plpro protease inhibitor of the virus.

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Conflict of interest: None declared

Ethical approval: Not Required

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