



Suppressing SARS-CoV2 Genome Replication: A Way to Overcome the Rate of Spread

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Abstract

One of the main reasons for the high prevalence of SARS-CoV2 is the high speed of its replication and reproduction. The replication inhibitors are under investigation due to the importance of prevention of the spread of coronavirus disease 2019 (COVID-19). In coronavirus replication, the virus enters the cell by endocytosis. After uncoating, the positive-strand RNA is translated to produce the non-structural protein (NSP) precursors. These precursors are cleaved and form mature, functional helicase and RNA polymerase. A replication-transcription complex (RTC) is then formed. Targeting the various stages of this process may be useful in preventing the spread of this epidemic. According to the similarity of the COVID-19 replication to the other single-stranded RNA viruses such as HCV, Ebola Virus, and Marburg, the best way to prevent the spread of infection is the viral genome replication targeting with specific drugs after exposure to the virus. For COVID-19 medications, compounds that target SARS-CoV2 replication are being tested in silico, in vitro, or in vivo. According to other clinical trials that have been applied for SARS-CoV and MERS-CoV inhibitor drugs in the attachment, protease, and replication stages can prevent the virus from multiplying. By reviewing previous related articles in this field, in this review article, we have tried to focus on all the information related to genome replication and categorize known drugs that have been applied as clinical trial treatments. The use of these drugs and other medications seems to be effective in reducing the prevalence of COVID-19.

Keywords: COVID-19, SARS-CoV2, Drug, Genome Replication, Spread

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Introduction

Human coronaviruses such as SARS-CoV and MERS-CoV, and recently SARS-CoV2, have been important pathogens with wide outbreaks worldwide. As COVID-19 caused a high rate of mortality and morbidity, there is an urgent requirement to explore and develop efficient and sufficient therapies and effective vaccines against COVID-19.¹ Since the prevalence of SARS and MERS (Coronaviruses with high homology to SARS-CoV2), several types of researches in this family of viruses have identified numerous suitable antiviral targets. The most critical viral proteins that have been targeted are viral proteases, polymerases, and entry proteins. As COVID-19 is extremely contagious and spreads quickly, the therapeutic strategies to deal with the infection seems to be only supportive, and prevention is reducing transmission.² During the coronavirus replication processes, the virus enters the cell by endocytosis. After uncoating, ORF1a, and ORF1b of the positive-strand RNA are translated to produce the nonstructural protein (NSP) precursors. Among nonstructural proteins, nsp1–nsp11 encoded in ORF 1a, and nsp12–16 encoded in ORF1b.³ These precursors are cleaved by protease and form mature,

functional helicase and RNA-dependent RNA polymerase. A replication-transcription complex (RTC) is then formed. More copies of the RNA genome are made via a negative-sense RNA intermediate. The next step in the coronavirus lifecycle is the viral genome encodes structural and other proteins. Finally, the viral RNA is packaged into viral coats in the endoplasmic reticulum-Golgi intermediate complex, leads to the release of viral particles from infected cells by exocytosis.⁴ The spike protein creates a crown that appears on the outer surface of the virion by forming a trimetric shape, which facilitates SARS-CoV2 replication. This protein leads to the entrance of the virus into the host cell.⁵ The interaction of the S1 and S2 domain of the spike and, its host receptor mediates attachment and fusion of the host cell and viral membrane resulting in RNA genome entering the host cell.⁴ In the coronaviruses family, the E-protein as a variable and the smallest transmembrane structure protein has two distinct domains. The E-protein plays a vital role in viral morphogenesis during assembly and CoV virulence.^{4,6} The M-protein is essential for the shape of the coronavirus envelope. The M-protein interacts with other proteins to

stabilize the nucleocapsid protein, which are involved in viral assembly (by an interaction between M–M, M–S, and M–N proteins) and viral intracellular homeostasis.^{4,7} The N-protein is a conserved protein in the coronavirus family and has been indicated that it has a vital function to maintain viral RNA binding to its complex proteins and its replication.⁸ According to the genome properties of coronaviruses (single-strand RNA viruses), they are similar to other single-stranded RNA viruses such as hepatitis C virus (HCV), HIV, Ebola virus, Marburg virus, dengue virus, West Nile virus, and rhinoviruses. For instance, coronavirus and HCV are both positive-sense, single-strand RNA viruses, and have a similar replication mechanism requiring an RNA-dependent RNA polymerase (RdRp).⁹ Due to the importance of this virus genome replication in

pathogenesis and the fact that vaccines have little use after exposure to the virus, the best way to prevent the spread of this infection is the virus genome replication targeting specific medicines. In this study, we review the therapeutic targets and drugs that may be available or those which have been predicted to prevent the SARS-CoV2 infection spread. The reason for the high prevalence of COVID-19 is its high replication potential. Lack of specific drugs for COVID-19 treatment and the time it takes to determine the results of the designed vaccines are the main challenges in COVID-19 treatment. It seems that using the existing drugs that can target the replication process are effective in controlling the prevalence of COVID-19. Figure 1 shows the situations of the replication process that have the potential to be effective by drugs.

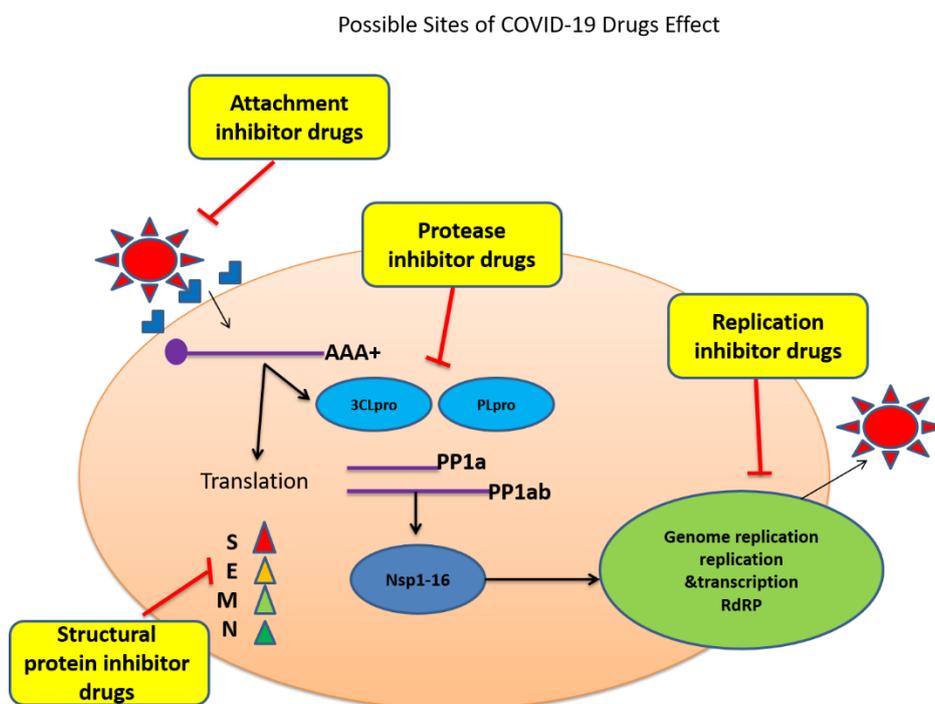


Figure 1. Different Stages of Virus Replication Processes Can be Targeted with Different Drugs.

Steps of the Life Cycle of SARS-CoV2

These processes contain four steps; after attachment, the second step is uncoating and releasing of viral RNA into the cytoplasm, the third step is translation of open reading frame 1a and 1ab which leads to produce the polyproteins pp1a and pp1ab. Processing of 16 non-structural proteins by viral proteases leads to form the RNA-replicase-transcriptase complex. In final step, the full-length (-) RNA copies of the genome synthesizes. These copies provide templates for full-length (+) RNA genomes. The transcription procedure produces a subset of subgenomic RNA, including all structural proteins. In the last step, the mature viral particles egress. To describe SARS-CoV2 replication, here we summarized the

proteins of coronaviruses, which have been investigated in different studies and can be targeted by drugs. Two large ORFs, rep1a, and rep1b, are encoded by the replicase gene. They express two co-terminal polyproteins, pp1a and pp1ab. Polyproteins pp1a and pp1ab contain the nsps 1–11, and 1–16, respectively, which are subsequently cleaved into the individual nsps by viral proteases.¹⁰ Nsp3 encodes papain-like protease (PLpro), and nsp5 encodes the main protease (Mpro). Nsp1 and nsp2 are formed as a multidomain complex by nsp3 and nsp5.¹¹ Nsp3 blocks the host's innate immune response and also promotes cytokine expression.¹² Nsp7 (may serve as a processivity clamp for RNA polymerase) and Nsp8 (may act as a primase processivity clamp for RNA

polymerase) form a hexadecameric complex that produces primers which is utilized by the primer-dependent nsp12 RdRp.^{13,14} Nsp9 acts as an RNA binding protein.¹⁵ Nsp10 is a scaffold protein for nsp14 and nsp16, which plays a role in exoribonuclease activity and methyltransferase function. Nsp15 has been known for endoribonuclease activity and the evasion of dsRNA sensors.^{4,16}

Drug Affecting Replication Processes

When a viral disease occurs suddenly, there are few or no effective treatments or vaccines available right away. For COVID-19, drugs and compounds that target SARS-CoV2 replication are being tested *in silico*, *in vitro*, or *in vivo*, and according to other clinical trials that have been applied for SARS-CoV and MERS-CoV.¹⁷ Jingyue et al. recently demonstrated that HCV drug EPCLUSA which is a combination of sofosbuvir and velpatasvir should inhibit coronaviruses, including SARS-CoV2. This group performed experiments *in vivo* using model polymerase extension. They confirmed that the active triphosphate form sofosbuvir blocked incorporation by low fidelity polymerases of SARS-CoV RdRp. Alovudine and AZT as two approved drugs for HIV were also evaluated, and their inhibitory function on SARS-CoV RdRP has been proved. Therefore, based on 98% amino acid similarity between SARS-CoV and SARS-CoV2, it is expected that these drugs would also be useful on SARS-CoV2.⁹ Another polymerase inhibitor used to treat the Ebola infection, remdesivir is developed in a clinical trial for treating SARS-CoV2.^{18,19} One of the crucial inhibitors for 3C-like protease (3CLpro) in coronaviruses is N-benzotriazole-1-yl-N-(benzyl) acetamido phenyl carboxamides. However, ML300 and ML188 are reported as some other CLPro inhibitors.^{20,21} Lopinavir and ritonavir (inhibitor of HIV protease) have been recognized as inhibitors of Mpro. Studies performing *in silico* showed a list of available drugs that bind to the lopinavir, and ritonavir binding site on coronaviruses. Among them are colistin, valrubicin, bepotastine, epirubicin and other commercial medicines.²² Interestingly, a high dose of zinc and zinc conjugates is an inhibitor of SARS-CoV proteases (Clpro and PLpro).²³ Sixteen candidates have been recently proposed for 3CLpro inhibition by Yu et al. in 2020. Ladipasvir and velpastavir are used as therapeutics to overcome COVID-19 with fewer side effects. Additionally, EPCLUSA (velpatasvir and sofosbuvir) and Harvoni (ledispavir and sofosbuvir) showed to be useful to inhibit viral enzymes (proteases and polymerase).²⁴ In 2011, Pfefferle used a system biology approach to identify the interaction partner of CoV nsp1. This researcher demonstrated that immunophilins interact with nsp1. The inhibition of cyclophilin by cyclosporine A (CspA) blocked the replication of CoVs as well as SARS-CoV.²⁵ Furthermore, α -ketoamide is known as a potential compound which is capable to inhibit SARS-CoV2 Mpro. This has been determined via x-ray

crystallization and is suitable for administration by the inhalation route.²⁶ In a recent study, an annotation result in NSP proteins for ORF1ab has been released by NCBI, and nsp6 has been considered as the only difference and as a putative protein that can be targets of pharmaceutical agents like protease inhibitor.²⁷ Other drugs used to target non-structural SARS-CoV2 proteins are listed in Table 1.

Discussion

Several studies have been published to identify the viral structure, route of transmission, and investigate likely vaccines and drugs to prevent COVID-19. Investigation for proprietary medicines against SARS-CoV2 is ongoing in *in silico*, *in vitro*, *in vivo*, and rarely clinical trial levels. As there is conserved protease in coronaviruses (96.1% the similarity of SARS-CoV with SARS-CoV2), drugs may be capable of preventing replication and proliferation of SARS-CoV2. Given the 98% amino acid identity of the SARS-CoV and SARS-CoV2 RNA dependent RNA polymerase, it also offers guidance to apply anticoronavirus agents or modify potentially nucleoside analogs.⁹ To date, there are no specific vaccines or anti-viral therapeutic targets for SARS-CoV2, and usual drugs for similar viruses have been tested so far. For example, scientists determined that nsp10/nsp16 referred to as methyltransferase or MTase is a potential target for the drug in SARS-CoV as well as SARS-CoV2.²⁸ Jingyue Ju1 et al. have mentioned that because coronaviruses and HCV are both positive-sense and single-strand RNA viruses, they have a similar replication mechanism requiring RNA-dependent RNA polymerase (RdRp).⁹ For another example, a vaccine based on the M-protein of SARS has been applied *in vivo* (mice), which showed induction and proliferation of T-cell in the immune response.²⁹ A cytotoxic T-cell response against SARS-DNA was also observed *in vitro* (in transfected cells). Occasionally, different types of immunotherapy for coronaviruses were used. For example, a study revealed that Interferons (IFNs) partially affect coronaviruses.³⁰ Stockman et al. demonstrated that IFNs combined with ribavirin in comparison to IFNs alone against some coronaviruses, might have increased activity *in vitro*. Further evaluation is required to investigate the effectiveness of this combination *in vivo*.³¹ The most important drugs used for targeting structural proteins are against ACE2, S, E, and N-proteins. Some antibodies against ACE2 can block the entry of CoV and its replication *in vitro*.³² It was also demonstrated that a peptide sequence with similarity to the receptor-binding domain or RBD of S-protein prevents the entry of SARS-CoV *in vitro*.³³⁻³⁵ Chloroquine as an antiviral agent inhibits SARS-CoV via interfering with the virus receptor binding. Recently, Kruse et al. performed experiments based on providing a soluble version of the viral receptor, ACE2, which blocks SARS-CoV2 entry. This version fuses the Fc domain of ACE2

Table 1. Drugs Used in Targeting Non-Structural Proteins (Especially the Genome Replication Process) of COVID-19

Protein (Enzyme)	Function	Usable for Viruses	Drug	Drug Mechanism
Nsp12	RNA-dependent RNA polymerase (RdRp)	SARS-CoV	Sofosbuvir	Nucleoside analog inhibitor
Nsp12	RNA-dependent RNA polymerase (RdRp), RNA-dependent RNA polymerase (RdRp), Clpro & Plpro (proteases)	SARS-CoV-2	Alovidine and AZT (for HIV/AIDS) EPCLUSA (hepatitis C) (Sofosbuvir/Velpatasvir)	SARS-CoV RdRp Pyrimidine nucleotide analogue prodrug protease inhibitor
ORF1a (RdRp, 3C-like proteinase)	Is Cleaved to protease and RNA dependent RNA polymerase	MHV (mouse hepatitis virus) CoVs	E-64D	cysteine proteinase inhibitor (RNA synthesis inhibitor). Inhibition of ORF1a cleavage and subsequently inhibition of RdRp cleavage
Nsp5	Protease	HCV, SARS, MERS	Ribavirin	viral replicase inhibitor (Synthetic guanosine nucleoside). Protease inhibitor
Nsp12	RdRP	SARS-CoV	Polyethyleneglycol-modified interferon- α	reduce viral replication and viral antigen expression
Unknown	Unknown	HCV		
mRNA	Protein translation	SARS-CoV MERS-CoV COVID-19	miR-4778-3p miR-6864-5p miR-5197-3p	complete complementary miRNA (cc-miR) binding sites in the gRNA coronaviruses without targetting human genes and suppress reproducing of the virus in the blood (experimental test)
Nsp12	RdRP	SARS	siRNA(s)	Gene targets for: RNA-dependent RNA polymerase, Helicase, proteolytic enzyme
Nsp12	RNA-dependent RNA polymerase	SARS-CoV-2	Remdesivir (GS-5734)	RNA polymerase inhibitor, nucleotide analogue prodrug
Nsp12	RNA-dependent RNA polymerase	Ebola, MERS Ebola, Influenza A(H1N1) COVID-19	Favipiravir (T-705)	Nucleoside analog: Viral RNA polymerase inhibitor
S proetin	Binding to cell receptor (ACE2)	SARS SARS-CoV-2 (treatment for Malaria)	Hydroxychloroquine	Change glycosylation of ACE2, inhibit viral entry
Unknown in CoV	Unknown in CoV	HBV, HCV Rotavirus, Norovirus, Coronaviridae	Nitazoxanide	broadly amplifying cytoplasmic RNA sensing and type I IFN pathways prevent viral replication
S protein	A receptor binding to ACE2 of host cell	Japanese encephalitis virus COVID-19	camostat mesylate	Blocking entry of virus and block infection of human epithelial cells in culture
3Clpro	a protease for the proteolysis of viral polyprotein into functional units	HIV/AIDS SARS MERS Not Effect on COVID-19	Lopinavir/ Ritonavir	Protease inhibitor
Nsp5	Proteases, 3C-like protease (3Clpro)	HCV SARS-CoV, COVID-19	EPCLUSA (valpatasvir and sofosbuvir)	Protease inhibitor
Nsp12	RNA-dependent RNA polymerase	SARS-CoV MERS-CoV HCV COVID-19	Harvoni (ledipasvir and sofosbuvir) Sofosbuvir, IDX-184, Ribavirin, and Remdisvir	Inhibition of polemerase function
Nsp1	Cellular mRNA degradation, inhibiting of IFN signaling	SARS-CoV	Cyclosporine A (CSPA)	Blocks coronavirus replication
Mpro	Protease	SARS-CoV2	α - ketoamide inhibitor	Protease inhibitor

As shown in the table, some of these drugs are not specific to COVID-19 but due to the similarities between this virus and other viruses, are usable for COVID-19. Some drugs that listed in the table are in the prediction phase (*in silico*), certain are in experimental phase (*in vitro* or *in vivo*), and various are used for patients.

immunoglobulin and offers a neutralizing antibody in order to avoid any viral escape. This ACE2 therapy also decreases ACE levels in the lungs during infections.³⁶ As an E-protein blocker, hexamethylene amiloride inhibits ion channel activity of E-protein *in vitro*.³⁷ Several inhibitors were designed to prevent the N-protein function, such as a chemical compounds that interact with the N-terminal end

of the N protein. Among these compounds, ribonucleotide 5'-monophosphatase (AMP, UMP, CMP, and GMP) interact and inhibit RNA binding of CoV.¹¹ Another study revealed that polyphenolic herbal compounds such as ectechingallate and gallactocatechingallate also can inhibit N protein of CoV.³⁸ C-terminal tail peptide sequence N377-389 competes with the C-terminal end of the N-protein to

inhibit c-terminal oligomerization and decrease the viral titer in vitro.³⁹ N220, as a nucleocapsid protein-peptide, has a high binding affinity in human MHC-1 in vivo (T2 cells). This peptide potentially selective nucleocapsid protein-expressing cells in the transgenic animal can be used in DNA vaccines.³⁹ In this paper, we review drugs against

non-structural proteins or those which are involved in SARS-CoV2 genome replication. However, it is necessary to design multiple drugs to treat COVID-19 as this infection remains for a long period of time. Some main medications for COVID-19 treatment has been tested in various levels of clinical trials as well as computer studies (Figure 2).⁴⁰

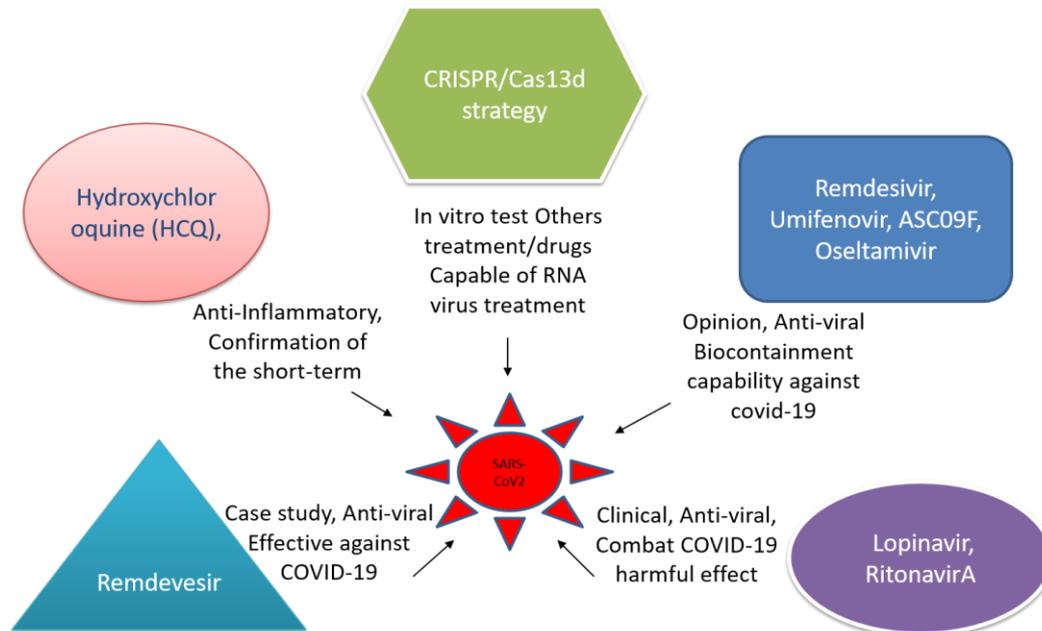


Figure 2. Several Drugs are being Studied at Different Stages to Treat COVID 19. Some of the Most Important Drugs are Listed in this Figure.

Conclusion

Neither vaccines nor therapeutics are specifically available against COVID-19. However, the knowledge of host cell proteins that take part in pivotal virus-host interactions could define the broad-spectrum antiviral targets. The identity similarities between coronaviruses and SARS-CoV2 have provided more benefits to figure out how research can be directed to cope with COVID-19. To design and develop drugs that inhibit the replication process of SARS-CoV2 and combat the COVID-19 outbreak, so many attempts remain to be examined and our knowledge about SARS-CoV2 updates every day.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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