Perspectives for repurposing drugs for the coronavirus disease 2019

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The newly emerged 2019 novel coronavirus (CoV), named as severe acute respiratory syndrome CoV-2 (SARS-CoV-2), like SARS-CoV (now, SARS-CoV-1) and Middle East respiratory syndrome CoV (MERS-CoV), has been associated with high infection rates with over 36,405 deaths. In the absence of approved marketed drugs against coronaviruses, the treatment and management of this novel CoV disease (COVID-19) worldwide is a challenge. Drug repurposing that has emerged as an effective drug discovery approach from earlier approved drugs could reduce the time and cost compared to de novo drug discovery. Direct virus-targeted antiviral agents target specific nucleic acid or proteins of the virus while host-based antivirals target either the host innate immune responses or the cellular machineries that are crucial for viral infection. Both the approaches necessarily interfere with viral pathogenesis. Here we summarize the present status of both virus-based and host-based drug repurposing perspectives for coronaviruses in general and the SARS-CoV-2 in particular.

Key words Coronavirus - COVID-19 - drugs - host-based - repurposing - severe acute respiratory syndrome coronavirus 2 - virus-based

Introduction

Coronaviruses (CoVs) belong to the family Coronaviridae and are enveloped, single-stranded, positive-sense RNA viruses¹. The CoVs are seen to be distributed in mammals as well as in humans causing mild infections. However, the severe acute respiratory syndrome CoV (SARS-CoV) and the Middle East respiratory syndrome CoV (MERS-CoV) from zoonotic sources in 2002 and 2012, respectively, were responsible for high infection and mortality rates². A novel CoV named as SARS-CoV-2, causative agent of the CoV disease 2019 (COVID-19), has caused 750,890 confirmed cases globally with 36,405 reported mortalities³. The SARS-CoV-2 belongs to the beta CoV genus which also includes the SARS-CoV-1 and the MERS-CoV. The lack of approved effective drug therapeutic protocols for CoVs would be a challenge for the treatment of the newly emerged COVID-19 infections worldwide.

Drug repurposing, which is defined as identifying alternative uses for approved or investigational drugs outside their defined indication, could be a possible
way to overcome the time limitation of research and development needed to design a therapeutic drug to combat the pathogen. Apart from having a lower risk of failure, most repurposed drugs have cleared phase I trials and require lower investment, but above all, the drug repurposing strategy drastically reduces the time frame for development. The drug repurposing or repositioning approach thus can facilitate prompt clinical decisions at lower costs than de novo drug development. Though drug repurposing is sometimes based on chance observations, target-based repurposing of drugs depends on prior understanding of the precise molecular or cellular element that is recognized by the proposed drug. The target may or may not essentially have the same mechanism of action in both the diseased states. Antivirals that can target the viral proteins or the key events in the viral life cycle, including virus-host cell interactions, replication, assembly and egress, would belong to this class. Drug repurposing to identify candidate drug compounds centred on the target-based criteria can thus be generally distinguished into virus- and host-based therapeutics. This review outlines the present status of both virus-based and host-based drug repurposing evaluations against the CoVs. The focus would be on the Food and Drug Administration (FDA)-approved marketed drugs or those under clinical trials against the CoVs in general, and the SARS-CoV-2 in particular.

Virus-based drug repurposing for coronaviruses

Virus-based antiviral agents target specific proteins of the virus. The major open reading frame, ORF1ab, of the SARS-CoV genome encodes the large replicase polyprotein pp1ab which forms the non-structural proteins, nsp1-16, while the structural proteins include S, E, M and N. The viral replication is facilitated by a replicase complex that involves processing of pp1ab by two cysteine proteases, namely the main protease (Mpro) or the 3C-like protease (3CLpro) and the secondary papain-like protease 2 (PL2pro) (Figs 1 and 2). Mpro cleaves at 11 sites in the central and C-terminal regions, while PL2pro cleaves at three sites in the N-terminal regions of the polyprotein. Majority of the proteins and enzymes of CoVs vital for the replication process are potential drug targets.

Main protease (Mpro)/ 3CLpro inhibitors - Lopinavir and/or lopinavir-ritonavir, cinanserin, herbacetin, rhoifolin and pectolinar

The Mpro is a promising viral target for the design of drugs against SARS/MERS, as the polyprotein cleavage by the Mpro facilitates the formation of the RNA-dependent RNA polymerase (RdRp) and the helicase which are the major proteins of viral replication. Various classes of protease inhibitors, such as halomethylketones, phthalhydrazide ketones, α, β-epoxyketones, glutamic acid and glutamine peptides with a trifluoromethylketone group, zinc or mercury conjugates, C2-symmetric diols, peptidomimetic-α, β-unsaturated esters, aldehydes, anilides, nitriles, pyrimidinone and pyrazole analogues, benzotriazole, N-phenyl-2-acetamide and biphenyl sulphone, are reported to inhibit the SARS-CoV-1 Mpro/3CLpro (Fig. 2). Of these prospective Mpro inhibitors, the common FDA-approved ones are well-known HIV-1 protease inhibitors. Among these, lopinavir and/or a ritonavir-boosted form of lopinavir has been reported to have anti-CoV activity in vitro and also has shown improved outcomes in non-human primates infected with MERS-CoV and in non-randomized trials with SARS patients. Both lopinavir and ritonavir are under phase II/III clinical trials for MERS-CoV (NCT02845843). These are also reported to have activity against HCoV-229E, HCoV-NL63 and animal CoVs.

Cinanserin (SQ 10,643) is a serotonin antagonist, demonstrated antiviral activity against SARS-CoV-1, and the inhibition of replication was probably by blocking the activity of Mpro. flavonoids, herbacetin, rhoifolin and pectolinar that are known to possess antioxidant effects associated with diseases such as cancer, Alzheimer’s disease and atherosclerosis were also noted to efficiently inhibit SARS-CoV-1 Mpro.

Papain-like protease (PLpro) inhibitor - Disulfiram

Disulfiram, which is an approved drug for the treatment of alcohol dependence, demonstrated in vitro inhibition of the PL2pro enzyme of SARS and MERS. The study also provided future directions for the development of fragment-linked inhibitors for improving its potency.

RNA-dependent RNA polymerase (RdRp) inhibitors - Ribavirin, immucillin-A/ galidesivir, remdesivir and acyclovir

The RdRp which is critical for CoV transcription and replication is involved in producing the genomic and subgenomic RNAs. Nucleoside analogues such as favipiravir, ribavirin, penciclovir, remdesivir and galidesivir are well-known RdRp inhibitors. A guanosine analogue, ribavirin, showed broad-
spectrum antiviral activity against several viruses including respiratory syncytial virus, hepatitis C and E viruses (HCV, HEV), chikungunya and viral haemorrhagic fevers. Though the mechanism of action is not fully understood, it is hypothesized that the drug may be involved in the inhibition of mRNA capping or viral RNA synthesis. The in vitro antiviral activity of ribavirin was demonstrated against SARS-CoV-1 and MERS-CoV and in rhesus monkeys infected with MERS-CoV. The drug has been used in the treatment of SARS and MERS patients, though the benefits are ambiguous. Further, in severely infected CoV patients, there could be side effects associated with high doses.

Immucillin-A (galidesivir), an adenosine analogue, has been shown recently as a broad-spectrum RdRp inhibitor against several RNA viruses, such as paramyxoviruses, flaviviruses, togaviruses, bunyaviruses, arenaviruses, picornaviruses, filoviruses and also against SARS/MERS-CoVs. Though it has been reported as a treatment option during the 2014-2016 West Africa Ebola virus epidemic, no data for animal/human were reported for CoVs until recently for the SARS-CoV-2.

Sheahan et al. showed that another nucleoside analogue, remdesivir (GS-5734), presently under clinical trials for the Ebola virus, demonstrated inhibition of the replication of SARS-CoV-1 and
MERS-CoV in primary human airway epithelial cells. They also demonstrated broad-spectrum anti-CoV activity against bat-CoVs and human CoVs in primary human lung cells. In another recent study, remdesivir was shown to possess better in vitro antiviral efficacy against MERS-CoV in comparison to lopinavir and ritonavir. In mice, remdesivir improved pulmonary function with lower viral loads in the lungs both as a prophylactic and as a therapeutic.

Another nucleoside analogue, acyclovir that was modified by incorporating fleximers to increase its binding affinity has been reported to be effective in vitro against MERS-CoV and HCoV-NL63, though to the best of our knowledge, no animal or human data are available.

**Inhibitors of spike glycoprotein - Griffithsin**

CoVs possess a surface structural spike glycoprotein (S) which is vital for interaction with the host cell receptor and subsequent virus entry into the cell. The S protein constitutes two subunits, the S1 (receptor-binding) and the S2 (membrane fusion) domains. Griffithsin, a lectin extract red algae, has been reported to bind to oligosaccharides on the surface of various viral glycoproteins, including HIV glycoprotein 120 and SARS-CoV glycoproteins.
Other inhibitors with unknown site of action - Resveratrol, amodiaquine, mefloquine, loperamide

Resveratrol, a natural compound from grape, which is in a clinical phase for heart and other diseases, was also reported to effectively inhibit MERS-CoV in vitro by downregulation of the apoptosis induced by the virus\(^\text{42}\). The possible site of action was suggested to be the nucleocapsid protein. Amodiaquine and mefloquine, antimalarial drugs, were also found to be effective against MERS-CoV\(^\text{43}\). Loperamide, an antidiarhoeal agent that was identified by the screening of an FDA-approved compound library, showed in vitro antiviral activity against MERS\(^\text{44}\).

Inhibitors of viral nucleic acids - Mycophenolic acid

Viral nucleic acids are mainly composed of nucleosides and nucleotides. The drugs that target these have mycophenolic acid (MPA) as the active compound and inhibit inosine monophosphate dehydrogenase and guanine monophosphate synthesis\(^\text{45}\). Broad-spectrum activity has been reported by MPA against a broad range of viruses including orthohepadnaviruses (hepatitis B), flaviviruses (HCV), arboviruses and CoVs. MPA possessed anti-MERS-CoV activity in vitro, though it was shown to result in a worsened outcome in the marmoset primate model\(^\text{55}\). Treatment of renal transplant recipients with MPA resulted in severe MERS\(^\text{56}\). Combination therapy with interferon beta-1b (IFN-β-1b) was, however, reported to be synergistic in vitro\(^\text{57}\), implying that monotherapy with the drug might not be useful for treating CoVs.

Host-based drug repurposing for coronaviruses

Specific host factors are utilized by CoVs for entry and replication. The anti-CoV potential of monoclonal antibodies (mAbs) evoked against the receptor binding domain (RBD) of S1 subunit and fusion inhibitors which target the S2 subunit has been reported in in vitro and/or in vivo studies\(^\text{46-50}\). SARS-CoVs and HCoV-NL63 preferably utilize the angiotensin-converting enzyme 2 (ACE2) host receptor while dipeptidyl peptidase 4 (DPP4) is used by MERS-CoV\(^\text{51,52}\) for entry. The further entry of CoVs into host cells includes the cell surface and/or endosomal pathways which are via host proteases such as transmembrane protease serine 2 (TMPRSS2) that cleave and activate viral S protein\(^\text{53}\). Inhibitors of these host proteases can prevent this proteolytic cleavage, partially blocking cell entry. Further, a group of drugs can target the endocytosis or cell entry\(^\text{44}\) (Fig. 2).

The innate IFN response of the host also has therapeutic potential as it controls viral replication after infection\(^\text{48,54}\). Additional pathways of cell signalling have also been noted as possible therapeutic targets for CoVs\(^\text{55}\). These classes of inhibitors are discussed below.

Inhibitors targeting endocytosis or cell entry - Chlorpromazine, ouabain, bufalin, chloroquine

Chlorpromazine, an antipsychotic/tranquilizer drug, is also known to affect the assembly of clathrin-coated pits at the plasma membrane\(^\text{44}\). It showed broad-spectrum in vitro activity against viruses such as HCV, alphaviruses, SARS-CoV-1 and MERS-CoV. Ouabain and bufalin, examples of a class of steroids which bind sodium- or potassium-transporting ATPase subunit α1, also inhibited the endocytosis of MERS-CoV mediated by clathrin. However, very high EC\(_{50}/C_{\text{max}}\) (half-maximal effective concentration value/peak serum concentration level) ratios at the typical dosages or toxicity, limit the clinical use of these endocytosis inhibitors. Acidification of the endosome can also affect endocytosis. Chloroquine, an antimalarial drug, can increase the intracellular pH by directing protons into the lysosomes\(^\text{57}\). It possesses broad-spectrum in vitro antiviral activities against flaviviruses, HIV, Ebola, Nipah and numerous CoVs\(^\text{58}\). However, it did not show activity in SARS-CoV-infected mice\(^\text{59}\). The anti-CoV activity of different endocytosis inhibitors thus need further in vivo evaluation.

Inhibitors of host receptor mediated viral entry - N-(2-aminoethyl)-1-aziridine-ethanamine (NAAE), peptides, mAb YS110

Specific peptide inhibitors and monoclonal or polyclonal antibodies can be used to target the host receptor\(^\text{48}\). N-(2-aminoethyl)-1-aziridine-ethanamine, a small-molecule inhibitor and synthetic ACE2-derived peptides showed inhibition of ACE2 activity and cell fusion via the S protein of SARS-CoV-1 in vitro\(^\text{60,61}\). However, these inhibitors have not been tested in CoV patients. Monoclonal antibodies (mAbs) such as anti-dipeptidyl peptidase 4 (DPP-4) have also been reported to block cell entry of MERS-CoV in vitro\(^\text{62}\). YS110, an anti-DPP4 recombinant humanized IgG1 mAb, used in a phase I clinical trial, was found to be well tolerated in patients with advanced malignancies\(^\text{19}\). However, considering that host cell receptor usage differs in different CoVs, the anti-CoV activity of these agents may be narrow-spectrum. Further, based on the vital biological functions of these receptors, the risks of immunopathology such as
Inhibitors of host proteases used for viral entry - Camostat mesylate, nafamostat

Camostat mesylate, a synthetic serine protease inhibitor, that is used to treat patients with chronic pancreatitis, works against the serine protease TMPRSS2. It has shown broad-spectrum activity against enveloped RNA viruses such as CoVs and paramyxoviruses. Camostat mesylate is reported to inhibit SARS and MERS in ex vivo studies and improves the survival of mice infected with SARS. Nafamostat, another serine protease inhibitor used to treat disseminated intravascular coagulation and pancreatitis, blocked MERS-CoV infection by inhibiting TMPRSS2 in human airway epithelial Calu-3 cells.

Enhancers of host innate immune response - Interferons, polyinosinic: polycytidylic acid [poly(I:C)] and nitazoxanide

Though on viral infection suppression of the IFN response is an integral part for immune evasion, several viruses and CoVs are noted to be susceptible to IFN treatment. The effectiveness of recombinant IFN-β over IFN-α has been demonstrated by in vitro studies against both SARS and MERS. IFN-α mediated reduction of viral titres was observed in SARS-CoV-infected in vivo models, while IFN-β administration via different routes was found to be effective in MERS-CoV in vivo models. Combinations of IFN-α/β, ribavirin and lopinavir/ritonavir-boosted lopinavir for treatment of SARS/MERS patients, demonstrated varying benefits.

Another type I IFN enhancer, polyinosinic: polycytidylic acid [poly(I:C)], a dsRNA synthetic analogue, demonstrated reduction in viral load in MERS-CoV-infected BALB/c mice. In phase II clinical trials, poly(I:C) was shown to be beneficial for patients suffering from malignant gliomas.

Nitazoxanide, a synthetic derivative of nitrothiazolyl-salicylamide which is used as a treatment for parasitic infections, is an effective type I IFN inducer. It has been shown to exhibit antiviral activities against several viral families and canine CoVs. Nitazoxanide was found to be safe in phase II and III clinical trials against HCV and influenza.

Inhibitors of signaling pathways involved in viral replication - Cyclosporine, trametinib and others

Drugs interfering with the viral replication signaling pathways are noted to have broad spectrum activity against several viruses such as HCV, HIV, vesicular stomatitis virus, human papilloma virus, vaccinia virus and CoVs. Cyclosporine, a calcineurin pathway inhibitor, inhibited a broad range of CoVs in vitro by interacting with the nsp1 protein and modulating immune response mediated by T cells. The clinical application of this drug is, however, restricted due to immune-suppressive effects and a higher EC50/Cmax ratio at standard dose levels. Other calcineurin inhibitors such as alisporivir, have demonstrated activity against HCoV-NL63.

The extracellular signal-regulated kinase (ERK) pathway mediates intracellular signals from membrane-associated Ras to the cytoplasmic kinase cascade Raf, Mek and Erk. The kinase signaling pathway inhibitors, such as trametinib (Mek inhibitor), selumetinib (Erk inhibitor), everolimus, rapamycin, dasatinib and imatinib have also demonstrated anti-CoV effects through inhibition of early viral entry or post-entry events. However, their toxicities may be a concern in severe infections.

Targeting viral translation - Silvestrol

Initiation of translation in many viruses happens through the usage of the host eukaryotic initiation factors (eIFs). The helicase eIF4A unwinds the 5′-untranslated region of the mRNA, facilitating assembly of the translation pre-initiation complexes. A natural compound, silvestrol, being an inhibitor of eIF4A and reported to show anti-cancer activity, demonstrated inhibition of MERS-CoV and HCoV-229E translation and replication in MRC-5 lung fibroblast cells.

Current perspectives for COVID-2019

Comparison of the coding regions of SARS-CoV-2 showed that it possessed a similar genomic organization when compared to bat-SL-CoVZC45 and SARS-CoV-1 (Fig. 2). Sequence analysis further revealed good sequence identity with the bat and human CoVs in the different coding regions. Except for the spike glycoprotein of SARS-CoV-2 that differs from the other CoVs including SARS-CoV-1 spike protein, the catalytic pockets in the
major non-structural viral enzymes are conserved at both the sequence and protein structural level across CoVs. Hence, repurposing of the promising MERS and SARS inhibitors for SARS-CoV-2 is a practical strategy\(^9\).

*In vitro* evaluations to test the antiviral potency of marketed drugs ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine and broad-spectrum RdRp inhibitors, remdesivir (GS-5734) and favipiravir (T-705) against SARS-CoV-2 were recently undertaken\(^8\). The findings have shown that remdesivir and chloroquine are more efficacious in comparison to the others. A patient from USA with COVID-19 who was treated with remdesivir intravenously was reported to have recovered\(^8\). Phase III trials (NCT04252664, NCT04257656) of intravenous remdesivir are currently ongoing to assess the efficacy in patients with SARS-CoV-2. Chloroquine is under an open-label trial for SARS-CoV-2 (ChiCTR2000029609). In addition, randomized clinical trials have been initiated for SARS-CoV-2 with favipiravir (ChiCTRChiCTR2000029544, ChiCTR2000029600) and ribavirin in combination with pegylated IFN (ChiCTR2000029387).

Results following rapid sequencing of the SARS-CoV-2, combined with molecular modelling based on homologous templates\(^8\) have identified certain compounds along with lopinavir and ritonavir that may be efficacious. Phase III clinical trials have also been initiated to test the HIV protease inhibitors including lopinavir (NCT04252274, NCT04251871, NCT04255017, ChiCTR2000029539), ritonavir (NCT04251871, NCT04255017, NCT04261270), darunavir and cobicistat (NCT0422274) in patients infected with SARS-CoV-2\(^21\). Another HIV protease inhibitor, ASC09F, in combination with oseltamivir is also in phase III clinical trial for SARS-CoV-2 (NCT04261270).

Arbidol (Umifenovir), a wide-spectrum antiviral drug inhibiting several flaviviruses and influenza viruses, whose mechanism of action is based on blocking crucial steps in virus- host cell interactions\(^8\), is under phase IV clinical trial for SARS-CoV-2 (NCT04260594, NCT04254874, NCT04255017). Oseltamivir, an influenza neuraminidase inhibitor\(^8\) is also under phase IV trial for SARS-CoV-2 (NCT04255017).

In the direction of host-based treatment strategies, randomized trials are underway for SARS-CoV-2 using recombinant IFNs (NCT04251871, ChiCTR2000029638)\(^9\). In another study, an artificial intelligence-based knowledge graph comprising systematically curated medical data, was searched for approved drugs against SARS-CoV-2\(^8\). Baricitinib, a janus kinase inhibitor, that was consequently identified, is a high-affinity AP2-associated protein kinase 1-binding drug which also interacts with a kinase regulator of endocytosis. Baricitinib has thus been suggested as a potential treatment for COVID-19 disease as it has the ability to reduce viral infection in lung cells.

**Molecular docking studies undertaken**

We analyzed the binding potential of HIV-1 protease inhibitors, lopinavir and ritonavir against the 3CLpro of SARS-CoV-2, using computational docking studies. This would help gain insight into the molecular mode of action of these drugs which are under clinical trials against the SARS-CoV-2 and also estimate the comparative inhibitory potency of the FDA-approved HIV protease inhibitors to the SARS-CoV-2.

The Mpro of CoVs cleaves substrates by recognizing the sequence motif (small)-X-(L/F/M)-Q↓(G/A/S)-X (X → any amino acid; ↓ cleavage site) and specifically the P1 site of the substrate requires a Gln (Q)\(^86,87\). The X-ray structure of SARS-CoV-1 3CLPro dimer bound with aza peptide epoxide (APE) as an inhibitor, (2A5K.pdb) was used for the modelling studies. The peptide showed major specificity to the S2 subsite and partial specificity to the S4 subsite of 3CLpro\(^12\). We detached the APE from the crystal structure complex and re-docked it computationally using the same protocol as for the two selected study inhibitors to obtain the docking score and it was found to be −8.27 Kcal/mol. The two inhibitors in this study had better binding potential (Fig. 3) when compared to APE. Comparison of the docked poses reveals that lopinavir occupies the S1' and S1 subsites with excellent complementarity while ritonavir occupies the S3 and S4 subsites with excellent complementarity through the benzene and 2' isopropyl thiozole groups respectively. These structural features indicate the possible mechanism by which these inhibitors can block the function of the SARS-CoV-2 3CLpro. The peptide substrate cleavage sites for SARS-CoV 3CLpro are noted to be at P1↓, P1' and P3↓P4\(^88,89\), the occupancy at the respective active site cavities would be crucial for competitive inhibition of the polyprotein substrate.
Based on this requirement, the findings are suggestive that ritonavir and lopinavir may have good potential for repurposing as SARS-CoV-2 protease inhibitors. Molecular dynamics simulation studies for the complexes obtained in this study would be essential to identify specific interactions between the enzyme and drug in the stable complexes and observe the hydrogen bond pattern, especially in the presence of solvent molecules. Additionally, studies need to be undertaken for the binding analyses of the other protease inhibitors, specific RdRp inhibitors and inhibitors of other enzymatic targets. The results would help gain an in-depth understanding of the relative binding affinity and design of derivatives with greater binding potential at the enzyme active site.

Conclusions

This review presented the information with respect to repurposing of FDA-approved drugs as well as those under clinical trials for SARS-CoV-1 and MERS-CoVs, wherein a lot of effort had gone in during the last decade or more. This knowledge has in fact, formed the basis for efforts towards drug repurposing for the SARS-CoV-2 as well. As highlighted in this review, phase III clinical trials of a few drugs have been initiated, though most of these are notably targeting the virus directly, essentially the RdRp or the chymotrypsin-like protease 3CLpro.

The spike glycoprotein also needs be explored as a target for the SARS-CoV-2 as the S1 domain of this virus deviates from the other human CoVs. It is thus important that the spike protein should be considered as a potential SARS-CoV-2 therapeutic target. On the other hand, considering that the strategy of targeting viral proteins is vulnerable to the emergence of viral resistance, other coronavirus targets such as the papain-like protease, helicase etc., also need to be attempted for drug repurposing. Further, several more of the potential SARS and/or MERS host-based inhibitors should be assessed against SARS-CoV-2. The ongoing vigorous efforts would help develop broad-spectrum anti-CoV agents against SARS-CoV-2.

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