INTERNATIONAL JOURNAL OF ADVANCED RESEARCH IN MULTIDISCIPLINARY STUDIES (IJARMS), VOL. 1, NO. 1, DECEMBER, 2021 **ISSN 2756-4444**
E-ISSN 2756-4452

REMDESIVIR: POTENTIAL REPURPOSED DRUG CANDIDATE FOR COVID-19

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Abstract
The outbreak of a new coronavirus severe acute respiratory syndrome– coronavirus 2 (SARS-CoV-2) has caused a global CoVid-19 pandemic, resulting in millions of infections and deaths around the world. There is currently no drug or vaccine for CoVid-19, but it has been revealed that some commercially available drugs are promising, at least for treating symptoms. Among them is Remdesivir, a drug that once offered hope against Ebola. SARS-CoV-2 is a single –stranded RNA virus – this means that it holds its genetic blueprint in a single strand of RNA. Every time the virus wants to replicate, it has to make a copy of this RNA. Remdesivir prevents this by giving the viral RNA copier (RdRP) fake RNA letters to use. Once SARS-CoV-2 docks on human cells using its spike protein and sneaks in, it can get the cells to make viral proteins based off of that RNA's instructions. However, human cells don't have the machinery needed to make copies of RNA from RNA. That task requires an RNA-dependent RNA Polymerase (RdRP) and the human cells only have DNA-dependent DNA Polymerase. Hence, before the virus can replicate, it has to get the human cells to make an RdRP using instructions in the viral genome which then has a daunting task of making sure that all of SARS-COv-2 genome letters are copied accurately each time the virus replicates. Remdesivir, which can block the activity of RdRP therefore becomes a potential therapeutic for COVID-19.
Keywords: SARS-CoV-2, Remdesivir, CoVid-19, RdRP

Introduction

In December 2019, a group of patients was admitted to hospitals with an initial diagnosis of pneumonia of an unknown etiology. These patients were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China (Bogoch *et al*., 2020; Lu *et al*., 2020). Early reports predicted the onset of a potential Coronavirus outbreak. Genomic sequencing showed that bat coronavirus was 96 % identical to this new coronavirus and shares 79.6 % sequence identity to SARS-CoV (Zhao *et al*., 2020). Given the estimate of reproduction the virus was called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease which it causes was referred to as Novel Coronavirus 2019 (COVID-19, named by WHO on Feb 11, 2020) (Zhou *et al*., 2020). The virus spread to almost entire globe in just 3 months. The infection was declared pandemic by World Health Organization (WHO) on March 11, 2020.

There are currently no specific antiviral drugs or clinically effective vaccines approved by FDA for the prevention and treatment of COVID-19 infections. However, the combination of a-interferon and the anti-HIV drugs lopinavir/ritonavir (Kaletra) has been used on the basis of drug repurposing theory to treat COVID-19 (Badgujar *et al*., 2020), but the curative effect remains very limited and there can be toxic side effects (Cao *et al*., 2020). Similarly, further research related to development of vaccine is ongoing but may take a long time to get a first effective and safe vaccine against COVID-19 (Singh *et al*., 2020). More recently, FDA has issued an emergency-use-authorization of Remdesivir, a broad-spectrum antiviral drug developed by Gilead Sciences Inc., for the treatment of COVID-19, but more data are needed to prove its efficacy (Cohen, 1999; Holshue *et al*., 2020; Wang, 2020). Indeed, specific anti–SARS-CoV-2 drugs offering efficacy and safety are urgently needed. In view of this, the present review article is aimed to highlight the therapeutic potential of Remdesivir.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

There are four different types of coronaviruses α, β, γ, and δ responsible for causing infection in humans and other vertebrate species. According to phylogenetic analysis, SARS-CoV-2 belongs to family Coronoviridae (genera Betacorona virus) that has previously caused two epidemics known as severe acute respiratory syndrome coronavirus (SARS-CoV) also known as SARS virus and middle East Respiratory Syndrome (MERS). The genome of SARS-SoV-2 was 96 % homologous to bat SARS like virus and also possess 79 % homology with SARS-CoV. In addition to this, pangolins have also been reported to be intermediate host of SARS-CoV-2 (Hui *et al*., 2020). World Health Organisation (WHO) has classified COVID-19 as a β CoV of group 2B (Lu *et al*., 2020). Ten genome sequences of COVID-19 obtained from a total of nine patients exhibited 99.98 % sequence identity (Ren *et al*., 2020). Another study showed there was 99.8 - 99.9 % nucleotide identity in isolates from five patients and the

sequence results revealed the presence of a new beta-CoV strain (Cui *et al*., 2019). The genetic sequence of the COVID19 showed more than 80 % identity to SARS-CoV and 50 % to the MERSCoV (Hui *et al*., 2020), and both SARS-CoV and MERS-CoV originate in bats (Cui *et al*., 2019). Thus, the evidence from the phylogenetic analysis indicates that the COVID-19 belongs to the genus betacoronavirus, which includes SARSCoV that infects humans, bats, and wild animals (Zhu *et al*., 2020). COVID-19 represents the seventh member of the coronavirus family that infects humans and has been classified under the *orthocoronavirinae* subfamily. The COVID-19 forms a clade within the subgenus sarbecovirus (Wan *et al*., 2020). Based on the genetic sequence identity and the phylogenetic reports, COVID-19 is sufficiently different from SARS-CoV and it can thus be considered as a new beta-coronavirus that infects humans.

The symptoms of COVID-19 infection appear after an incubation period of approximately 5 days (Li *et al*., 2020). The period from the onset of COVID-19 symptoms to death ranged from 6 to 41 days with a median of 14 days (Huang *et al*., 2020). This period is dependent on the age of the patient and status of the patient's immune system. It was shorter among patients > 70-years old compared with those under the age of 70 (Huang *et al*., 2020). The most common symptoms at onset of COVID-19 illness are fever, cough, and fatigue, while other symptoms include sputum production, headache, haemoptysis, diarrhoea, dyspnoea, and lymphopenia (Huang *et al*., 2020). Clinical features revealed by a chest CT scan presented as pneumonia, however, there were abnormal features such as RNAaemia, acute respiratory distress syndrome, acute cardiac injury, and incidence of grand-glass opacities that led to death (Wang *et al*., 2020). In some cases, the multiple peripheral ground-glass opacities were observed in subpleural regions of both lungs (Cui *et al*., 2019) that likely induced both systemic and localized immune response that led to increased inflammation. Regrettably, treatment of some cases with interferon inhalation showed no clinical effect and instead appeared to worsen the condition by progressing pulmonary opacities (Cui *et al*., 2019). It is important to note that there are similarities in the symptoms between COVID-19 and earlier betacoronavirus such as fever, dry cough, dyspnea, and bilateral ground-glass opacities on chest CT scans (Wang *et al*., 2020).

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses. The genomic RNA of coronaviruses is ~30,000 nucleotides in length with a 5′-cap structure and a 3′-poly(A) tail, and contains at least six open reading frames (ORFs) (Hussain *et al*., 2005; Chen *et al*., 2020). The first ORF (ORF 1a/b), about two-thirds of the genome length, directly translates two polyproteins, pp1a and pp1ab, so named because there is an a-1 frameshift between ORF1a and ORF1b. These polyproteins are processed by a main protease, Mpro [also known as the 3C-like protease (3CLpro)], and by one or two papain like proteases, into 16 nonstructural proteins (NSPs). These NSPs engage in the production of sub-genomic RNAs that encode four main structural proteins {envelope (E), membrane (M), spike (S), and nucleocapsid (N) proteins} and other accessory proteins (Ramajayam *et al*., 2011; Ren *et al*., 2013). The replication and transcription are facilitated by the assembly of non-structural proteins (nsp), which are produced as a result of the cleavage of viral polyproteins encoded by open reading frame 1a (ORF1a) and ORF1b (Ziebuhr *et al*., 2005; Chen *et al*., 2020). Established RNA-dependent RNA polymerase (RdRp or also known as nsp12) play a crucial role in the replication and transcription of SARS-CoV-2 virus because it catalyzes the synthesis of viral RNA. Like SARS-CoV, the entry of SARS-CoV-2 into the host cell begins by attaching the S protein on the virial surface to the Angiotensin-converting enzyme 2 (ACE2) of the host cell (Kuba *et al*., 2005; Nguyen *et al*., 2020). Once SARS-CoV-2 docks on host cells using its spike protein and sneaks in, it can get the cells to do a lot of its wok for it – like making viral proteins based off of that RNA's instructions –but the host cells don't have the machinery needed to make copies of RNA from RNA. That task requires an RNA-dependent RNA polymerase (RdRP) but the human cells only have DNA-dependent DNA polymerase (which is used for copying its own genome) and DNA-dependent RNA polymerase (which we is used to make mRNA copies of genes from which protein is made). So, before the virus can replicate, it has to get the human cells to make an RdRP using instructions in the viral genome.

RNA-dependent RNA polymerase (RdRP) Function and Structure

RNA-dependent RNA polymerase (RdRP) also known as RNA replicase is one of the most versatile enzymes that is indispensable for catalyzing the replication of RNA from an RNA template. Specifically, it catalyses synthesis of RNA strand complementary to a given RNA template. This is contrary to typical DNA-dependent RNA polymerases, which all organisms use to catalyze the transcription of RNA from a DNA template. RdRP is an essential protein encoded in the genomes of all RNA-containing viruses with no DNA stage (Zanotto *et al*., 1996; Koonin *et al*., 1989). The core structural features of RdRps are conserved, despite the divergence in their sequences. All RNA viruses and some DNA viruses encode RdRPs that facilitate viral gene transcription and replication in concert with other viral and host factors (Gorbalenya *et al*., 2002).

There are 4 super families of viruses that cover all RNA-containing viruses with no DNA stage. They include positive-sense RNA, segmented negative-sense RNA, non-segmented negative-sense RNA and dsRNA (Tan *et al*., 1996). The structure of RdRP resembles that of a cupped right hand and consists of fingers, palm and thumb subdomains. Five of the seven classical RdRP catalytic motifs (A–E) are located within the most conserved palm domain, while the other two (F and G) are within the finger domains (Gorbalenya *et al*., 2002; Venkataraman *et al*., 2018; Gao *et al*., 2020). The structurally conserved RdRP core and the related motifs are essential for viral RdRP catalytic function. The catalysis involves the participation of conserved aspartates and divalent metal ions. Complexes of RdRps with substrates, inhibitors and metal ions provide a comprehensive view of their functional mechanism and offer valuable insights regarding the development of antivirals (Venkataraman *et al*., 2018).

Although substrate requirements differ, all characterized RdRPs share the same catalytic mechanism. On infection of the host cell, viral RdRP participates in the formation of the genome replication machinery by complexing with other factors (Ahlquist, 2002). RdRP initiates and governs the elongation of the RNA strand that includes the addition of hundreds to thousands of nucleotides. The RNA replication process is a two-step mechanism. First, the initiation step of RNA synthesis begins at or near the 3' end of the RNA template by means of a primer-independent (de novo), or a primer-dependent mechanism that utilizes a viral protein genome-linked (VPg) primer. The de novo initiation consists in the addition of a nucleoside triphosphate (NTP) to the 3'-OH of the first initiating NTP. During the following so-called elongation phase, this nucleotidyl transfer reaction is repeated with subsequent NTPs to generate the complementary RNA product (Kao *et al*., 2001; Jin *et al*., 2012). Nucleotide analogs can stop the RNA elongation catalyzed by RdRP once they are inserted into the newly synthesized RNA chain.

Remdesivir an Inhibitor of RNA-dependent RNA polymerase (RdRP)

Remdesivir was originally developed in 2009 by GILEAD pharmaceutical for the treatment of hepatitis, but it exhibited moderate anti-viral potency against hepatitis (Stephens, 2020). It has also been used against various coronaviruses and for treatment of Ebola in 2014 which displayed efficient activity against the Ebola virus (Warren, 2016). Remdesivir is a monophosphoramidate prodrug of an adenosine analog (GS-441524) that works by inhibiting the basic viral RNA replication process inside the host-cell and causes termination of a broad-spectrum viral activity against several viral families such asparamyxoviruses, filoviruses and coronaviruses (Murphy *et al*., 2018; Tchesnokov *et al*., 2019; Choy *et al*., 2020). Remdesivir (GS-5734) acts as a pro-drug of actual remdesivir (GS-441524). GS-5734 is primarily metabolized into GS-441524. It is administered in an inactive form whereby it undergoes different stages of enzymatic reaction. Hence, GS-5734 is converted to GS-441524which is an active form of triphosphate nucleotide (Eastman *et al*., 2020). Remdesivir has two hydroxyl groups, which may become orally active through chemical modification by masking one of them as an ester. The ester group gets hydrolyzed by esterase enzyme which then leads to produce carboxylic acid derivative and corresponding carboxylate ion (Tchesnokov *et al*., 2019). This carboxylate ion attacks on phosphorous group and leads to form cyclic phosphor-anhydride with release of phenoxide ion. Then cyclic phosphor-anhydride ring get open into the phosphate ion alanine metabolite.

Finally, this alanine metabolite gets hydrolyzed and gets converted into the nucleoside monophosphate by reaction of enzyme phosphoramidase. This nucleoside monophosphate gets converted into the nucleoside triphosphate and nucleoside analogue GS-441524 acts as an active metabolite which incorporated in the anti-viral mechanism which is a modified nucleo-base similar to that of adenosine (Al-Tannak *et al*., 2020). On cell entry, remdesivir converts to a nucleoside triphosphate (NTP). Studies have confirmed that remdesivir-TP competes with adenosine triphosphate (ATP), the natural nucleotide, for incorporation into the nascent RNA strand (Amirian *et al*., 2020; Gordon *et al*., 2020) and acts as an alternative substrate for the purified RdRP complex. The incorporation of remdesivir-TP halts the growth of the RNA strand. The mechanism of its inhibitory effect is categorized as delayed RNA chain termination owing to the fact that such inhibition does not occur immediately after the incorporation of inhibitor.

Conclusion

The current review summarizes the known knowledge on the development of remdesivir as an RNA-dependent RNA polymerase (RdRP) inhibitor. The apparent development of effective anti-SARS-CoV-2 medications in a short period of time involves significant hurdles and unknown risks.

Recommendations

The following recommendation were made from the study:

1. However, research and development and clinical trials of other drugs in addition to the potential antiviral candidate medication remdesivir, are also underway.
2. Researchers from academic institutions and pharmaceutical companies, as well as front-line physicians, should work together more closely to boost pharmaceutical preclinical and clinical studies of anti-coronavirus medications.

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