Review Article

Pathogenesis of SARS-CoV-2 and Important Insights on its Potent Inhibitors Remdesivir and Chloroquine - A Review

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, single-stranded, positive-sense RNA virus which is responsible for coronavirus disease 2019 (COVID-19) characterized by pulmonary infection in humans. Effective prophylactic and therapeutic management for COVID-19 are urgently required to control this pandemic. Angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2 in host cell. Spike protein of virus binds with the peptidase domain of ACE2 receptor. After binding with the receptor the next step of virus is to gain entry to the host cell cytosol which is accomplished by the proteolytic cleavage of S protein in acidic pH. The proteolytic cleavage is followed by fusion of SARS-CoV-2 and its host cell membrane leading to release of the viral genome into the cytoplasm. The antimalarial drug chloroquine and its analog hydroxy-chloroquine are known to restrict viral replication by increasing endosomal pH which inhibits viral-cell fusion. After uncoating, viral RNA genome is translated to form structural viral protein with the help of RNA-dependent RNA polymerase (RdRp), which is the target for adenosine analogue remdesivir. The combination therapy including Remdesivir and chloroquine are exceptionally successful in the control of 2019-nCoV disease in vitro. Fundamental preliminaries of Remdesivir and chloroquine repurposing in the treatment of COVID-19 have been empowering, prompting a few new trials. In this review, pathogenesis of the SARS-CoV-2 and the potential mechanism of two potent inhibitors remdesivir and chloroquine as effective therapeutic agents against COVID-19 are described.

Keywords

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Introduction

The novel Corona Virus Disease 2019 (COVID-19) is a global pandemic which emerged in Wuhan City of China in December 2019, and currently affected more than 200 countries while posing a high threat owing to rapidly increasing number of cases and deaths on daily basis (Chan et al., 2020; Chatterjee et al., 2020; Marty and Jones, 2020; WHO, 2020). Coronaviruses belongs to betacoronavirus (β-CoVs or Beta-CoVs) genus (Zhou et al., 2020), family Coronaviridae, of enveloped, single-stranded, positive-strand non-segmented RNA viruses. The name “coronavirus,” was coined in 1968, which means crown-like morphology which was seen in the electron microscope (Tyrrel,
1968). The clinical sign is that of a respiratory disease with a manifestation ranging from a mild cold-like sickness, to an extreme viral pneumonia prompting intense respiratory trouble disorder that is potentially fatal. Clinical preliminaries and in-depth examinations to study the infection pathogenesis, its origin starting point and gaining transmission and spread to humans, and how it influences people are progressing. Contrasted with SARS-CoV and MERS-CoV, COVID-19 infection displays quicker human-to-human transmission, hence prompting WHO to assertion of an overall general well-being crisis (Chan et al., 2020).

Since, COVID-19 is spreading like havoc in form of pandemic across the countries, prompt prophylactic and therapeutic measures are required. The development of new drugs and vaccines demands sufficient time for production, pre-clinical, clinical trials, manufacturing and validation, therefore to meet the requirement repurposing of drugs is a smart strategy and need of the current situation.

With the help of knowledge of SARS-CoV-2 virus architecture and pathogenesis, target sites can be identified for proposing drugs which can impose anti-viral activity with pronounced safety and efficacy (Garcia-Serradilla et al., 2019; Rosa and Santos, 2020).

Although, specifically efficacious drugs against the SARS-CoV-2 are yet to be established the chloroquine and hydroxychloroquine have been considered as a potential therapeutic option to treat COVID-19. The chloroquine phosphate is reported to be the first drug to display efficacy against SARS-CoV-2 in China (Gao et al., 2020). Due to easy availability and low cost of the chloroquine and hydroxychloroquine these drugs may prove crucial to curb the ongoing pandemic after proper evaluation of its efficacy and safety by clinical trials. A nucleotide analog viz. remdesivir which was reported to be administered in Ebola virus patients has also proven effective against coronavirus (Costanzo et al., 2020). The present article describes the pathogenesis of SARS-CoV-2 and gives insights on its two potent inhibitors Remdesivir and Chloroquine for use as effective drugs against COVID-19.

**Pathogenesis of SARS-CoV-2**

SARS-CoV-2 virus has three envelope proteins which help the virus to enter into the host cell viz the spike (S) protein, the envelope (E) protein and the membrane (M) protein. Beside this virus also have nucleocapsid (N) which is an important element of the replicase complex. First interaction of virus to the host cell is started by associations between the S protein and its receptor.

On account of SARS-CoV, the spike glycoprotein (S protein) on the SARS-CoV-2 surface recognizes receptor and mediates viral and host cell membrane fusion (Gallagher et al., 2001; Simmons et al., 2013). When virus attacks, the trimeric structured S protein is cleaved into S1 and S2 subunits.

S2 subunit is responsible for the post fusion conformation (Simmons et al., 2013; Song et al., 2018). S1 subunit has the region for receptor binding, which straight forwardly binds with the angiotensin-converting enzyme 2 (ACE2) at its peptidase domain (PD) (Li et al., 2005), while S2 is liable for fusion of membranes. When S1 subunit binds with ACE2 receptor on the host surface another cleavage site S2 is uncovered and is targeted by host proteases, a procedure which is important for viral spreading (Millet et al., 2015; Simmons et al., 2005). The S protein-receptor binding is the prime step of
coronavirus to infect a host. The receptor used by SARS-CoV-2 to enter the host is ACE-2 (Li et al., 2003). The next step of virus is to gain entry to the host cell cytosol. This is accomplished by the proteolytic cleavage of S protein by cathepsin, TMPRSS2 or any other protease requires acidic environment for the cleavage that is not possible under physiological pH. The proteolytic cleavage is followed by fusion of SARS-CoV-2 and its host cell membrane leading to release of the viral genome into the cytoplasm (Bosch et al., 2003). Following uncoating, the positive sense RNA genome is released which is translated to large polyprotein that experiences proteolytic processing to form a RNA-dependent RNA polymerase.

Through the activity of the RNA polymerase, a full-length, antisense negative-strand template is formed. The subgenomic negative sense templates give rise to subgenomic negative-sense mRNAs which are further translated to form structural viral proteins. Following replication, the envelope proteins are translated and embedded into the endoplasmic reticulum and afterward move to the Golgi compartment. Viral genomic RNA is bundled into the nucleocapsid and afterward envelope proteins are integrated to form mature virions. The M protein, which restricts to the trans-Golgi network, assumes a fundamental job during viral assembly by associating with different proteins of the virus. Following assembly, the viruses are carried to the cell surface within vesicles and are discharged by exocytosis (Devaux et al., 2020). The discharged virus can infect different cells and replicate inside the parent cell through binding to CEACAM-1 (Bergmann et al., 2006; Shereen et al., 2020). These viral particles reach to bronchi, alveoli, and extrapulmonary organs, and causes pneumonia which are the main cause for death.

**Remdesivir and chloroquine as potent inhibitors of SARS-CoV-2**

**Remdesivir**

The RNA dependent RNA polymerase also called as Nsp12 plays an important role in the replication and transcription of SARS-CoV-2. Therefore, Nsp12 is viewed as a key target for antiviral inhibitors. The drug targeting RNA-dependent RNA polymerase (RdRp) of SARS-CoV could be effective in treatment with SAR-CoV-2 because SARS-CoV-1 have 82% RNA sequence resemblance, and 96% sequence resemblance of RdRp with SARS-CoV-2 (Liu et al., 2020) (Figure 1).

Remdesivir, a wide acting anti-viral drug molecule could likely be beneficial for the treatment of COVID-19 (Wang et al., 2020; Holshue et al., 2019; Rabaan et al., 2020). Remdesivir is a 2-ethylbutyl L-alanine phosphoramidateprodrug (Warren et al., 2016) that has wide antiviral property against various families of virus in vitro (Lo et al., 2017), including the respiratory syncytial virus, Hendra virus, Ebola and Nipah virus in non-human primate models (Warren et al., 2016; Singh et al., 2018; Lo et al., 2019). Remdesivir (referred with code GS-5734™) is as adenosine analog, prodrug of an adenosine C–nucleoside (Ko et al., 2020). More precisely, remdesivir (GS-5734) is phosphorylated from GS-441524 (Amirian and Levy, 2020) and is 3-30 times more active than GS-441524 (Menachery et al., 2015). Studies have shown that remdesivir inhibit replication of MERS-CoV and SARS-CoV and has also proved it efficacy in pre-pandemic Bat-CoVs, and human-CoV virus in epithelial cells of airway in human which indicates that remdesivir likewise could hinder the replication of a wide range of coronaviruses (Sheahan et al., 2017; Al-Tawfiq et al., 2020).
Remdesivir, an adenosine analogue, works after the entry of the virus into the host cell. After entering the host cell it get metabolized into nucleoside triphosphate (NTP) which competes with ATP and lowers the replication of human as well as in zoonotic delta coronaviruses by inhibiting replication of RNA (Brown et al., 2019). It gets integrated in the viral RNA chains and results in RNA chain termination. This implies that the viral RNA dependent RNA polymerase introduces adenosine analog rather than the natural nucleotide (Agostini et al., 2018). It adds three additional nucleotides and afterward stops due to pre-mature termination of RNA replication, as viral exonuclease does not work properly and due to faulty proof reading of nucleotide chain, replication of viral RNA could not be completed (Agostini et al., 2018). As a result, the virus can't replicate its genome; it cannot reproduce and make its host diseased. The researchers hypothesize that the additional three nucleotides may shield the drug from being expelled by the coronavirus' exonuclease enzyme (Gordon et al., 2020).

Due to beneficial anti-viral effects remdesivir has also been tried along with interferon beta and have demonstrated promising, protective and safe effects against MERS-CoV in murine model (Cao, 2020). In pre-clinical studies the broad-spectrum anti coronavirus activity of remdesivir was demonstrated and for evaluation of safety and efficacy of remdesivir in hospitalized patients with mild to moderate COVID-19 a randomized, controlled, double blind clinical trial has already been designed (Cao, 2020).In vitro and in vivo both studies have confirmed favourable effects of remdesivir on animal models like transgenic mice and rhesus macaques against MERS-CoV (Amirian and Levy, 2020; de Wit et al., 2020). Remdesivir is presently being tried in five COVID-19 clinical preliminaries that have been set up very fast. A fundamental report on COVID-19 patients treated with the trial of remdesivir recommends that the antiviral drug may bring down the danger of death in seriously sick patients and improve the state of patients depending on breathing devices. Although, clinical impacts of remdesivir on COVID-19 patients are unknown the scientists are putting their best possible step forward and waiting patiently for the final results of ongoing clinical trials.

**Chloroquine**

Another widely used medication to treat SARS-CoV-2 is Chloroquine, as approved by the US Food and Drug Administration (FDA) despite lack of full evidence of its action and a few side effects as well (Devaux et al., 2020; Gao and Hu, 2020). It is a notable anti-malarial drug and immunomodulator which has been accounted as a potential broad spectrum antiviral medication (Savarino et al., 2006; Yan et al., 2013). The antiviral property of chloroquine and its derivatives against RNA viruses was earlier reported for SARS-CoV-1, rabies virus; influenza virus; Nipah virus; Chikungunya virus; polio virus and for many more (Devaux et al., 2020).

Chloroquine phosphate is now an approved drug compound possessing anti-SARS-CoV-2 activity and hence is recommended to be used against COVID-19 but under clinical supervision (Duan et al., 2020). Chloroquine and/or hydroxychloroquine any compound can be used, however hydroxychloroquine is recommended over chloroquine due to rapid GI absorption, reduced toxic effects, and wider safety spectrum, which can even be administered safely in pregnant patients (Liu et al., 2020). Moreover, hydroxychloroquine is a chloroquine analog considered as an immunomodulator and reported to have more potent in vitro inhibitory effect on SARS-CoV-2 than chloroquine (Yao et al., 2020;
The difference between chloroquine and hydroxychloroquine is that the N-ethyl substituent in chloroquine is β-hydroxylated in hydroxychloroquine (Devaux et al., 2020). In treatment regimen of hydroxyl-chloroquine, loading dose should be followed by maintenance dosage (Colson et al., 2020). Administration of hydroxyl-chloroquine depicted marked reduction in occurrence of serious fatal clinical symptoms of COVID-19 (Yao et al., 2020; Zhou et al., 2020). Chloroquine (CQ) is a 4-aminoquinolone derivative which is a weak base and can enter the cells and accumulate in endosomes, trans-Golgi network vesicles or lysosomes like acidic compartments raising cell pH (Thome et al., 2013). Chloroquine is known to inhibit viral infection by increasing endosomal pH i.e making it alkaline and thus inhibiting endocytosis and viral-cell fusion which require acidic pH. By altering the pH, chloroquine also affects the post-translational modification of viral proteins (Devaux et al., 2020). It blocks the action of pH dependent enzymes like glycosyltransferases or proteases in golgi network which impedes the maturation of viral protein (García-Serradilla et al., 2019). It interferes with the M protein maturation which in turn effect the virion assembly and replication (Randolph et al., 1990). It also affects the glycosylation of ACE2 cell receptors of SARS-CoV. Other than its antiviral action, chloroquine is a known drug for autoimmune disease, which may also synergistically improve its antiviral impact in vivo. The SARS-CoV-2 affected patient’s shows increase in cytokines such as interleukins and other inflammatory agent which causes cell apoptosis, as well as a cytokine storm.

Chloroquine is known to alter these cytokine which can cause havoc in the body and thus helps in body defence. Chloroquine was reported to have an inhibitory effect on the production and release of cytokines like IL-6 and TNFα and may prove crucial in suppression of the COVID-19 associated cytokine storm (Gao, et al., 2020). Hydroxychloroquine suppresses the activity of T-cells to regulate the cytokine storm in the body. Chloroquine affects the immune system not only by regulating the cytokines but also via affecting MAPK signaling pathways in THP-1 cells and caspase-1 (Steiz et al., 2003). The MAPK signaling is required by the virus for its replication (Briant et al., 1998). Beside attachment of SAR-CoV-2 with protein membrane receptor, the virions can also bind with the gangliosides and sialic acid containing glycoprotein in the respiratory tract (Matrosovic et al., 2015). Chloroquine has higher binding affinity with these receptor in comparison with SARS-CoV-2, and thus prevent attachment of virus to the cells of pulmonary system to check the viral entry (Fantini et al., 2020). The inhibitory impacts of chloroquine on virus can be before or after the virus entry thus proposing both its prophylactic and therapeutic role.

Recently a study hypothesized, consequential and synergistic action of hydroxychloroquine and ivermectin for chemoprophylaxis and clinical care of COVID-19 patients on simultaneous administration (Patri and Fabbrocini, 2020). However, in a pilot study chloroquine was reported superior to lopinavir/ritonavir in decreasing the period of hospitalization and improving the pulmonary radiological appearance (Gao and Hu, 2020; Huang et al., 2020). In addition, adverse effects reported to be associated with chloroquine includes nausea, vomiting, diarrhea, abdominal pain, rashes, itching, cough and shortness of breath (Gao and Hu, 2020; Huang et al., 2020). The clinical study on hydroxychloroquine to treat COVID-19 revealed reduction in period of fever, decrease in the duration of cough and improvement in lung images suggesting the usefulness of the drug in management of COVID-19 (Chen et al., 2020).
Notwithstanding the notable elements of chloroquine, increasing the endosomal pH, the medication seems to affect the terminal glycosylation of ACE2 cell receptor for SARS-CoV-2 (Gao et al., 2020; Vincent et al., 2005). This may adversely impact the virus-receptor binding and abrogate the infection, by the rise of vesicular pH, bringing about the hindrance in uncoating of virus (Figure 1) and spread of SARS-CoV-2 (Vincent et al., 2005; Faiq et al., 2020).

Chloroquine is generally effective, safe and cheap medication utilized for treating numerous human illnesses including malaria, human immunodeficiency virus and amoebiosis and is compelling in hindering the disease and spread of SARS-CoV-2 in cell culture. The clinical trial for chloroquine prophylactic and therapeutic effect against SARS-CoV-2 has started in United States.

Research performed in Central China’s Hunan Province, South China’s Guangdong Province and in Beijing hospital, China revealed that combined therapy of chloroquine and remdesivir effectively reduce the SARS-CoV-2 activity under in vitro conditions and they recommended use of this drug-combination for treating COVID-19 infected patients (Hu et al., 2020). Chinese medical advisory board has proposed addition of chloroquine in the treatment prescribed for SARS-CoV-2 (Wang et al., 2020).
The fast worldwide spread of COVID-19 infection has stressed the requirement for the advancement of new coronavirus immunizations and therapeutics. In this context, researchers already started exploring all possible remedies and combinations for containment of this pandemic. Many antivirals, protease inhibitors and supplementary pharmaceuticals were considered and used for treatment and chemoprophylaxis of earlier and ongoing coronavirus disease.

Although, no therapeutic agent provided promising result in case of ongoing pandemic but chloroquine and remdesivir were considered effective on the basis of many studies and in vitro trials. The viral polymerase RdRp looks a key target for new therapeutic remdesivir which is a nucleotide analog and prevents replication of viral genomic RNA. The ability of anti-malarial drug chloroquine and its analog hydroxychloroquine to raise the endosomal pH and hence inhibiting the cell fusion and release of viral genome into the cytoplasm is also explored for containment of SARS-CoV-2 infection. The Chinese medical advisory board has also recommended the inclusion of chloroquine in the treatment prescribed for SARS-CoV-2 infection. Many in vitro studies suggested that remdesivir and chloroquine may prove highly useful and effective in the control of COVID-19 infection.

The combination therapy including chloroquine and remdesivir was found highly effective in reduction of the SARS-CoV-2 activity under in vitro conditions. In addition, this drug combination is recommended for the use in treatment of COVID-19 patients. The utilization of these drugs in human patients demonstrated their effectiveness against different ailments; we propose that they ought to be assessed in human patients experiencing the novel coronavirus illness.

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