REVIEW

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Recent advances of therapeutic targets and potential drugs of COVID-19

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Since December 2019, numerous cases of coronavirus disease 2019 (COVID-19) caused by the infection of the novel coronavirus (2019-nCoV) have been confirmed in Wuhan, China. The outbreak of 2019-nCoV in China embodied a significant and urgent threat to global health. 2019-nCoV was a new, highly contagious coronavirus discovered following the outbreak of SARS coronavirus (SARS-CoV) and MERS coronavirus (MERS-CoV). The novel coronavirus can cause severe respiratory disease and even death. However, no specific therapeutic drugs have been developed clinically thus far. This article examines the potential of therapeutic drugs by assessing the structure of 2019-nCoV, its mechanism in invading host cells, and the anti-viral mechanism of the human autoimmune system. We also review the latest research regarding the progress of potential therapeutic drugs and provide references for new drug developments of COVID-19.

1. Introduction

In December 2019, Wuhan, China experienced an outbreak of coronavirus disease 2019 (COVID-19) caused by infection of the novel coronavirus (2019-nCoV), which rapidly led to a pandemic in the following ensuing months (Shi et al 2020). As of March 10, 2020, data from the World Health Organization (WHO) showed there the COVID-19 outbreak had reached 110 countries with 113702 confirmed cases, which significantly affected the economic development of China and the world economy. Although the 7th Edition of the Pneumonia Diagnosis and Treatment Protocol for COVID-19 Infection (Trial Version) in China proposed drugs such as ribavirin, lopinavir, interferon, and glucocorticoids to treat COVID-19 in China, different therapeutic effects arose under different clinical treatments. In addition, to date, no vaccine has been approved worldwide to prevent COVID-19 (Zhao et al. 2020; Pang et al. 2020; Hoffmann et al. 2020). Therefore, this article examines the potential of therapeutic drugs by assessing the structure of 2019-nCoV, its mechanism in invading host cells, and the anti-viral mechanism of the human autoimmune system. We also explore suitable therapeutic targets and potential therapeutic drugs for COVID-19.

2. Potential therapeutic drugs related to 2019-nCoV structure

2019-nCoV, a positive-strand RNA virus, can encode at least four structural proteins, including the spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. The S protein, M protein and E protein are involved in the formation of viral coat and the N protein encapsidates the RNA genome. Fischer et al. (2020) screened 11 different novel lead compounds for these viral protease targets and virtual experiments showed that each compound had a small absorption and low binding free energy, indicating promising potential for drug development (Fischer et al. 2020). In addition, 2019-nCoV can also encode some functional proteins, such as viral papain like protease (PLpro), main protease (3CLpro, also named 3-chymotrypsin-like protease), RNA-dependent RNA polymerase (RdRp) and helicase, which can assist viral RNA replication, transcription and translation (Wu et al. 2020). Therefore, drugs that destroy 2019-nCoV structural proteins or inhibit 2019-nCoV functional enzymes can make the virus structure incomplete, prevent the replication and assembly of viral RNA, and inhibit its invasion of host cells.

Wu et al. (2020) used a computer aided drug design method to screen antiviral drugs (ribavirin, valganciclovir and thymidine), antibacterial drugs (chloramphenicol, cefamandole and tigecycline), and muscle relaxant drugs (chlorphenesin, carbamate) with a relatively strong affinity for papain-like protease (PLpro) protein, showing strong hydrogen bonding and hydrophobic forces. In addition, anti-asthmatic drug montelukast showed low binding energy to 3CLpro. Therefore, these drugs have potential to treat COVID-19 (Fischer et al. 2020).

In addition, RNA-dependent RNA polymerase (RdRp), a conservative protease of coronavirus responsible for the replication of viral RNA, is an ideal target because it does not exist in the human body. Gilead's sofosbuvir acts as an RNA polymerase inhibitor by competing with natural ribonucleotides to inhibit the RdRp of the hepatitis C virus. Ju et al. (2020) hypothesize that Gilead Science's sofosbuvir can be an RdRp inhibitor and a potential 2019-nCoV inhibitory drug. Remdesivir most probably targets RdRp and is an adenosine analog that can integrate into the nascent viral RNA strand, causing RNA to terminate prematurely. Recently, multiple clinical studies using Remdesivir to treat COVID-19 are underway. One study showed that remdesivir can effectively block 2019-nCoV infection at low micromolar concentrations *in vitro* (Wang et al 2020b). Although clinical trials of remdesivir have not been completed, the drug has been successfully applied in the United States to treat COVID-19. Therefore, parallel development of new drugs and old drugs can accelerate the speed of drug development and control the spread of COVID-19.

3. Potential therapeutic drugs related to 2019-nCoV invasion of host cells

Zhou et al. (2020) found that the pathway in which 2019-nCoV infected human cells is the same as that of severe respiratory

syndrome coronavirus (SARS-CoV), exemplified by the S protein of the 2019-nCoV combined with the angiotensin-converting enzyme receptor 2 (ACE2) on the surface of the host cell (Zhou et al. 2020). Therefore, a drug developed to inhibit the viral S protein and ACE2 receptor binding sites can lead to a promising treatment for COVID-19. Wu et al. (2020) used a computer virtual screening approach to predict that the anti-diabetic drug teglitazone, antihypertensive drug losartan, and analgesic glutamine can bind to ACE2 stably. However, for lung diseases, the loss of ACE2 enhances vascular permeability and lung edema, activates the renin-angiotensin system, and contributes to the pathogenesis of severe lung injury if there is no suitable interface site to inhibit binding to the viral S protein (Kuba et al. 2010).

Furthermore, Hoffmann et al. (2020) reported that 2019-nCoV can use both cysteine protease cathepsin B (CatB/L) and serine protease (TMPRSS2) to activate the S protein in cell lines. These two proteases need to be inhibited in order to prevent viruses from severely invading the body. Camostat mesylate is a clinically-proven serine protease inhibitor and a potential anti-SARS virus drug (Kawase et al 2012). It can block the driven entry of 2019-nCoV S protein into host cells by inhibiting the activity of TMPRSS2. E-64d, an inhibitor of CatB/L, can also play a potential role in blocking infections when used with camostat mesylate.

Another novel route in which 2019-nCoV invades host cells is by means of the CD147-spike protein (SP) and CD147, which is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily. Meplazumab, as a humanized anti-CD147 antibody, could competitively inhibit the binding of SP and CD147 as well as prevent viruses from invading host cells, thus having the potential of an antiviral drug (Wang et al 2020a).

In addition, the S1 subunit binds to cell receptor through its receptor binding domain (RBD) and subsequently, the S2 subunit undergoes a conformational change that inserts the fusion peptide into the host target cell membrane. In the S2 subunit, the heptad repeat 1 (HR1) region forms a homotrimeric assembly, which binds to the heptad repeat 2 (HR2) and helps bring the viral and cellular membranes into close proximity for viral fusion and entry. It has been reported that the HR1 region in the S protein is conserved among various human coronaviruses HCoVs, which could also be a good target for the development of fusion inhibitors against COVID-19 (Chen et al 2020b; Wrapp et al. 2020). Based on this target, the peptide fusion inhibitor EK1 can broadly inhibit a variety of HCoV infections, which is evident in HCoV-OC43 and MERS-CoV infected mouse models that have shown positive antiviral effects *in vivo* (Xia et al 2020). The peptide was found to have better safety and lower immunogenicity, thus showing the potential to be further developed into specific drugs for the prevention and treatment of the currently prevalent COVID-19 and also for new HCoVs that may emerge in the future. In summary, it is clear that the mechanism of 2019-nCoV invasion into host cells is the first step to seek drug targets. Only then, can new drugs be accurately developed to impede the manner that the virus infects the human body, rendering the virus to be incapable of reproducing and spreading.

4. Potential therapeutic drugs related to anti-viral mechanism of the human autoimmune system

The human autoimmune system plays a critical role in preventing the acute infection of this zoonotic virus, especially in the absence of specific drug treatment. When the virus enters the human body, the virus's structural and functional proteins can induce the production of a variety of antibodies within the body. However, the antibodies can only exert antiviral effects when it recognizes and binds to the virus particles. Some antibodies are directed at the viral protein in the envelope or in the host cell, but is unable to come into contact with the antigen and thus unable to achieve an antiviral effect.

The most direct treatment method to enhance the patient's immunity is to transfer the plasma of a recovered patient. Antibodies in the plasma have a direct impact on the virus with less side effects and can be utilized to treat critically ill patients. It may also be more effective to administer convalescent plasma at the early stages of the disease. However, quality of plasma antibodies depends on the plasma source. Other treatments may influence the relationship between plasma and antibody levels during recovery, such as antiviral drugs, steroids, and intravenous immunoglobulins. Therefore, it is essential to control the quality of plasma antibodies during recovery (Chen et al 2020a).

Tian et al. (2020) reported that a monoclonal neutralizing antibody, CR3022, that is specific to SARS-CoV can strongly bind to the receptor-binding domain (RBD) in 2019-nCoV and SARS-CoV. CR3022 can be developed as a candidate antibody drug, alone or combined with additional neutralizing antibodies (such as m396, CR3014), and it can be used to prevent and treat 2019-nCoV infection (Tian et al 2020). On the other hand, there is also a non-neutralizing antibody that mediates immune cell phagocytosis and clears infected cells after binding to the virus, but it also causes the virus to easily escape the immune system. Macrophages can be used to expand and promote inflammatory storms, especially fatal patients, and it is mostly caused by the infection of cells and inflammatory factors.

Corticosteroids are commonly used drugs that can inhibit lung inflammation, but also inhibit the immune response and the body's elimination of viruses (Russell et al. 2020). Chloroquine is a drug widely used against malaria and autoimmune diseases that not only blocks viral infection by increasing the *in vivo* pH required for virus/cell fusion, but also inhibits viral infections by interfering with the glycosyl groups of SARS-CoV cell receptors. Moreover, it also has immunoregulatory functions, especially in terms of suppressing the immune factor storm caused by 2019-nCoV virus. Wang et al (2020b) found that low concentrations of chloroquine phosphate can inhibit 2019-nCoV infection of human cells *in vitro*, which may not cause cytotoxicity, suggesting that chloroquine drugs may be employed in the treatment of COVID-19.

Some drugs used in Traditional Chinese medicine can nourish qi and protect evil spirits, disperse wind, remove heat, dispel dampness, regulate the physiological balance of the human body, and strengthen the human body's resistance, which has accounted for half of the clinical research in the treatment of COVID-19 (Zhang et al. 2020). Luo et al. (2020) reported that according to the historical records and clinical evidence of SARS and H1N1 influenza virus infection prevention, it is speculated that Chinese medicines such as Kangbingdu Oral Liquid can be important as a method to prevent 2019-nCoV infection in high-risk populations, but it is still necessary to confirm its treatment effect by carrying out reasonable compatibility and clinical experiments. Therefore, drugs that are used to enhance human immunity should be reasonably administered to avoid adverse effects.

5. Conclusion

Since the outbreak of 2019-nCoV, human health and life has been threatened globally. Unfortunately, the development of appropriate drugs is time-consuming and consists of numerous processes. The development of many novel drugs for SARS-CoV ceased to progress as the viral epidemic ended. However, unlike SARS, COVID-19 may become a chronic disease and coexist with humans, like influenza. For sudden viral infections, it is undoubtedly wise to screen for suitable treatments among existing drugs. Hence, this article reviewed various pathways in which the virus infects the human body and proposed potential therapeutic drugs. However, these drugs still have certain limitations, either with poor specificity or limited therapeutic effects. In the long run, the development of contemporary, broad-spectrum antiviral drugs to combat a wide range of HCoVs may become a treatment for COVID-19 and the ultimate treatment strategy in preventing emerging HCoV infections.

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