

COVID-19's replication and potential therapeutic drugs

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ABSTRACT: *This passage is based on the ongoing COVID-19 pandemic. The first part of this passage compares COVID-19 virus with normal influenza virus, summarizes the significant facts about COVID-19, and talks about COVID's viral proliferation process. The second part mainly explains 3 potential therapeutic drugs against COVID and proposes 2 other treatments. This passage is about 10 pages.*

KEYWORDS: *COVID Proliferation, Potential Drugs, Mortality, Trials' Results*

Background

Coronavirus Disease 2019, which is also widely known as the COVID-19 is an ongoing global pandemic that was first originated in the year 2019, caused by a virus called the Severe Acute Respiratory Syndrome Coronavirus II(SARS-CoV-2). Until July 13, 2020, the virus has been detected in more than 188 countries, causing 571000 deaths, and thus it has become the worst epidemic for the past ten years[1].

COVID-19 is a type of Coronavirus; Coronavirus is a kind of RNA virus that can only infect vertebrates like cows, chickens, pigs, humans etc. Scientists thought of the Solar Corona when they were looking at the image of Coronavirus and that's how the virus' name came from. The diameter of the coronavirus is about 80-120nm, the 5'end of the genome has a methylated cap structure, and the 3'end has a poly(A) tail. The full length of the genome is about 27-32kb. It is currently the largest RNA virus. The Coronavirus was first extracted from chickens in the year 1937. They can cause different symptoms on different animals, for instance, they can cause respiratory infection on chickens, but they cause diarrhea on cows and pigs. COVID-19 is the seventh Coronavirus that been found can infect humans. The remaining six other Coronaviruses are HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, and MERS-CoV[7].

Differences between COVID and Influenza

As the continuing rampant growth of COVID-19, scientists began to compare the difference between COVID-19 and the normal flus. Although both of them are categorized into respiratory diseases, but there are several significant differences on

how they spread as well as how they act out on patients respectively. Distinguishing these differences can also contribute a lot to find the potential treatment for COVID-19 and to help us getting a better understanding of viruses. But let's talk about the similarities between the two viruses for priority.

First of all, the patients who get infected by COVID-19 and flu virus have a similar symptom: they have trouble breathing. Since lungs are the most vulnerable organ to these respiratory viruses, in most cases, lungs will be attacked by these viruses first. When these viruses have successfully invaded patients' lungs, these viruses may cause severe shock and even death on patients. Second, both viruses can be spread through our daily contact, water droplets and secretions[4]. Thus, keeping hands clean and wearing masks in public places are important actions that we can take to prevent from getting infected. Now, let's talk about the difference between the two viruses.

The main distinction of the two viruses is the speed of transmitting. According to the World Health Organization: "Influenza has a shorter median incubation period (the time from infection to appearance of symptoms) and a shorter serial interval (the time between successive cases) than COVID-19 virus." [2]The serial interval of the influenza is about 3 days. While for COVID-19, the serial interval has been prolonged to 3~5 days. And this signifies that the influenza will be spread at a faster speed than COVID-19. Additionally, COVID-19 virus has a greater reproductive number. According to the World Health Organization, the number of secondary infections generated from one infected individual is understood to be between 2 and 2.5 for COVID-19 virus, which is higher than the influenza virus. Besides that, for COVID-19, adults are more affected than children. Study in China suggest that the death rate and attack rate during the 0~19 age group are low, most of the people who get infected are elder people. (Except for some cases in US, young people who were not aware of the pandemic often hang out with their friends and brought the virus back home and thus infected the oldsters in their family.) By contrast, most of the susceptible population to influenza are pregnant women and children. Another thing is the mortality for COVID-19 seems to be much more higher than influenza, higher for about 100~200 times. For COVID-19 virus, according to World Health Organization, crude mortality ratio (the number of reported deaths divided by the reported cases) is between 1-2%, the infection mortality rate (the number of reported deaths divided by the number of infections) will be lower. For seasonal influenza, mortality is usually well below 0.1%.

Most common symptoms of COVID-19 are fever, dry cough, and fatigue; the proportion of these three symptoms presenting on patients are 87.9%, 67.7%, and 38.1% respectively. There are also some other symptoms such as Sputum production, Shortness of breath, and sore throat etc. One of the most uncommon symptoms presenting on patients is Conjunctival congestion, only possesses a ratio of 0.8%[3].

COVID's Proliferation

2019-nCoV is a single positive strand RNA virus with a genome of about 30 kb. (If a virus' base sequence of viral RNA is exactly the same as that of mRNA, the virus is classified as a positive-strand RNA virus.) The genome is composed of two untranslated regions at both ends and multiple encoding regions for non-structural proteins (NSPs) and structural proteins. These structural proteins include spike protein (S), envelope protein (E), membrane protein (M) as well as nucleocapsid protein (N), and these structural proteins will do a great help for the virus' proliferation[5].

Virus' proliferation is called self-replication. This proliferation process can be executed only if the virus successfully enters human cells. The virus does not have a complete enzyme system, does not have the raw materials and energy to synthesize its own components, and does not have ribosomes, so lacking of these essential things have determined that the virus is congenitally parasitic. It must invade living cells and utilize the host cell's enzyme system, raw materials, and energy to replicate its own nucleic acid and translate its proteins. The virus' proliferation process(Replication Cycle) is consist of five steps, which are adsorption, penetration, uncoating, biosynthesis, and release[11].

The first step, adsorption means that the virus' superficial Spike proteins (S) attach to the human cells' protein receptors. According to this step, the host range of many viruses can be determined, and infection cannot be caused without adsorption. Virus adsorption is also affected by environmental conditions such as ionic strength, pH, and temperature. Studying the process of virus adsorption can have great significance for understanding the receptor composition, function, pathogenic mechanism and exploring antiviral therapy. Different viruses will attach on different receptors; for instance, HIV's receptor is CD4, Rhinovirus' receptor is cell adhesion molecule-1 (ICAM-1), and COVID-19's receptor is ACE2. So what exactly is a protein receptor? Take the COVID-19's receptor ACE2 as an example. According to Paul Insel, ACE2 is a kind of protein on the surface of many cell types, which is also a kind of enzyme that can produce smaller proteins by dividing a larger protein angiotensinogen and regulate the cell's function. The SARS-CoV-2 virus uses spike-like proteins on its surface to bind to ACE2 before entering and infecting cells (like inserting a key into a lock). Therefore, ACE2 acts as a gate-receptor for COVID-19. ACE2 can be found in many epithelial cells of organs in our body such as heart, lungs, kidneys, vessels etc. ACE2 will line in order in these organs and form a protection screen for us [6] [8].

The second step, penetration means the fusion of the two membranes—the virus' membrane and the human cell's membrane—which enables the capsid that wraps the virus' genetic materials to get into the cell. For example, those fusion proteins present on the envelope of measles virus and mumps virus. These proteins will carry a section of hydrophobic amino acid, which generates the fusion of cell membrane and virus envelope.

The third step, uncoating means the cracking of capsid; penetration and uncoating are both continuous processes, and the loss of the integrity of the virus body (capsid) is so called "uncoating". There is an enzyme presenting in human cells which is called Lysosome. Lysosomes are the major digestive chamber of cells. They comprise a variety of enzymes which are responsible for decomposing different types of biological materials, including nucleic acids, lipids, and proteins. They present in both animal cells as well as some types of plant cells (presenting in vacuoles), and can break down various types of macromolecules that are presenting in the cells. In most cases, these macromolecules are either dysfunctional or damaged; they have already finished their life cycle and are no longer useful to the cells. Lysosomes can degrade these cells after they die. Although they can be found in nearly all types cells of animals except for red blood cells, they are particularly ample in such tissues and organs that participate in high enzymatic reactions such as kidney, liver, pancreas and so on. There are mainly two types of Lysosomes, Primary Lysosome and Secondary Lysosome. The primary Lysosome contains hydrolase, it is a Lysosome that has not undergone digestion. Secondary Lysosomes (digestive vesicles) are formed by the fusion of Primary Lysosomes and phagosomes (produced by cell phagocytosis), and are Lysosomes that have been supplied with hydrolytic enzymes. During the uncoating process, Lysosome in human cells will release digestive enzyme to decompose the capsid of the virus. After that, the virus begin to release its genetic material and start to replicate itself. Many people may wonder why Lysosomes won't digest themselves; It is because before the lysosomes are exposed to certain signals that tells them to decompose, they are at their inactive state.; and thus lysosomes won't digest themselves[9].

The fourth step, biosynthesis means viral nucleic acid replication and viral protein synthesis. DNA viruses and RNA viruses have different replication mechanisms, but the result of replication is the synthesis of nucleic acid molecules and protein capsids, and assemble into a new infectious virus. A replication cycle may takes about several hours. Still, take the COVID-19 virus as an example. When SARS-CoV-2 has successfully underwent its uncoating process, it will first release a sense strand RNA(Positive strand RNA), and uses the host cell's ribosome to translate this sense strand RNA. Then, the virus will eventually get two proteins: pp1a and pp1ab. These two proteins rapidly degraded by proteolytic enzymes into structural and non-structural proteins, such as RNA-dependent RNA polymerase. Under the action of this enzyme, a double-stranded structure is formed using the parental RNA as a template, which is called a "replicative form". Then the virus will copy multiple progeny positive-strand RNA (Subgenomic mRNA) from the complementary negative strand. This structure composed of a complete negative strand and multiple positive strands that are growing is called a "replicative intermediate". The new progeny RNA molecules have three functions in the replication loop: they may act as a template for further synthesizing the replicator; may continue to function as mRNA; or may constitute infectious viral RNA. For SARS-CoV-2, the new progeny RNA molecules will first go through a translation and then constitute infectious viral RNA which will later become an entirely new virus continue to infect other cells[6].

The last step is called assembly and release, The process of combining newly synthesized viral nucleic acid and viral structural proteins into viral particles in the infected cell is called assembly, and the process of transferring the newly packed virus from the interior to the exterior is called release. RNA viruses mostly replicate nucleic acids and synthesize proteins in the cytoplasm. One cell can produce up to 10,000 virus particles, after six hours it was infected. After the virus has been successful assembled, there are two different releasing methods. The first one is the host cell will lysed, the virus will eventually released into the surrounding environment, which is found in non-enveloped viruses, such as adenovirus, poliovirus, etc. The second one is released by budding, which is found in enveloped viruses. For instance, Herpes simplex virus obtains an envelope on the nuclear membrane, influenza virus obtains an envelope on the cell membrane to mature, and then release mature virus by budding, the virus can also pass through cell bridges or fuse with adjacent cells. COVID-19 has an envelope; correspondingly it will release its newborn virus by the second way.

Potential Drugs

After knowing its proliferation process, the real problem is how to treat COVID-19.

Disappointedly, up to now there is no effective treatment recommended for COVID-19. Some people propose that Hydroxychloroquine, a disease-modifying anti-rheumatic drug (DMARD) used to treat Helopyra, which regulates our immune system can be generalized as the ‘most effective drug’ against COVID. Some researches done in 2004 and 2005 do claim that it is useful to inhibit the original SARS corona virus. However, this drug has been discontinued by WHO as well as other authoritative organization since no evidence can be made from the tests that Hydroxychloroquine can cause any effect on patients. After some critics cast doubt on the authenticity of Hydroxychloroquine, on June 4 2020, Lancet recalled a paper suggesting that the drug increased the mortality rate of COVID-19 patients. This discovery halted numerous ongoing test. However, a study in United Kingdom announced its result of the Hydroxychloroquine test on June 5. In a group of 1542 COVID patients that were treated with Hydroxychloroquine had a mortality rate of 25.7% after 28 days, while the control group of 3132 COVID patients received normal treatment had a mortality rate of 23.5%. This result unquestionably shows that there is no benefit from Hydroxychloroquine. Some scientists also agree on this result; “It just seems like we are ignoring signal after signal,” says Eric Topol, director of the Scripps Translational Science Institute. U.S. ‘President Donald Trump’s promotion of it led to a scientific “obsession” with hydroxychloroquine despite thin evidence for its promise,’ he says. “We’d be better off shifting our attention to drugs that might actually work.” Peter Kremsner of the University of Tübingen also agrees that hydroxychloroquine “certainly isn’t a wonder drug.” [10]

Although Hydroxychloroquine has been proven not a drug against COVID, but there may be some other potential drugs. The University of California Davis Medical Center in Sacramento, CA recently proposed a drug treating COVID-19,

which is called Remdesivir[11]. This drug is based on the principle that COVID-19 must first replicate itself in order to infect other cells. If the replication process is interrupted, then the virus is no longer minatory. In other words, this drug inhibits the virus to replicate and to copy its viral genome. Remdesivir is a broad-spectrum antiviral drug; its specific mechanism is to prevent the RNA-dependent RNA polymerase to combine the virus' RNA strand. RNA polymerase is a viral-encoded enzyme that helps the virus to replicate. By this, the enzyme is blocked and allows the immune system to initiate a stronger respond to fully eliminate the virus. Remdesivir has proven to have some positive effects against MERS-CoV (a relative coronavirus to SARS-CoV-2 on primates) and show vitro activity against SARS-CoV-2. The team conducted a treatment using Remdesivir to treat a female patient. This patient is one of the earliest cases of COVID-19 been recorded on United States mainland. Her doctor confirmed that the patient's situation was very severe before she was treated with Remdesivir. After the first day taking Remdesivir, she began to show significant improvement. Some of her blood samples have been frozen in order for researchers to do further tests. US and China are all doing numerous ongoing trials to test the feasibility of Remdesivir, but detailed results haven't come out yet. American Food and Drug Administration (FDA) passed an Emergency Use Authorization (EUA) about Remdesivir against COVID-19 on May 1, 2020. But it does not mean that the drug was officially approved by the American Government; Instead, FDA's purpose was to let doctors obtain Remdesivir more easily in order to treat those most severe patients.

Another potential drug is now being developed by the company Regeneron, which is Monoclonal Antibody[12]. This drug is based on the strategy that straightly attacks the virus itself. Inhibiting the virus' proliferating process (Adsorption, Penetration, Uncoating, Synthesis, Assembly and Release) can also prevent further infection. In fact, our immune system will done this by naturally producing various antibodies when exposing to virus. Antibodies are a type of protein that recognize and bind pathogens. When antibodies successfully bind these pathogens, they can destroy these pathogens and neutralize them. But COVID-19 is an entirely new virus, which means that those people who are not infected by COVID do not have antibody in their immune memory since they are not exposing to the virus. Nevertheless, those one who have recovered from COVID-19 have such antibodies. If we can extract these functional copies (Monoclonal Antibodies) and reproduce them, they can not only become a substitution to raise the tensity of immune system, but also to imitate the attack on COVID-19. Similar methods have been used on treating certain types of cancer; some trials have also been conducted in order to treat a variety of bacterias and viruses such as Escherichia coli, Clostridium difficile, HIV, respiratory syncytial virus (RSV) and rabies virus. Regeneron is now searching for ways to produce Monoclonal Antibodies in a large scale. This drug works by preventing those spike proteins attach on ACE2 receptor—blocking SARS-CoV-2's adsorption process. Regeneron has announced that they have already successfully extracted those Monoclonal Antibodies from COVID recovered patients. They also claim that they decide to conduct human-trials at the early summer.

The third potential drug is called Favipiravir, also known as Avigan[12][13]. This drug has been permitted in China and Japan to cure influenza. Recently, United States also permitted Favipiravir human trial in Boston. A recent vitro study also suggests that high doses of Favipiravir can prevent infection from COVID-19. Two researches in China are aiming to compare the effectiveness of Favipiravir treating COVID-19 with other antivirals. In a group of 240 patients with mild COVID-19 infection, 71% of the patients treated by Favipiravir recovered from the disease comparing with a rate of 56% for those who were given Umifenovir (Arbidol). Another ongoing trial in China which researched 80 mild infection patients with COVID-19 discovered that under the same condition, the speed Favipiravir takes to eliminate virus is faster than the speed of Kaletra. (4 days and 11 days respectively). Those patients who received Favipiravir treatment also showed great improvement of their lungs.

As far as I'm concerned, there are also other two ways that we can use to treat COVID. The first one is using Fusion Inhibitors. For instance, there is a kind of fusion inhibitor called Enfuvirtide (T-20). It is classified as a type of anti-AIDS targeted drug, and it consists of 36 amino acid residues. It was developed by Trimeris company in the United States and Roche company in Switzerland together. The annual therapy of Enfuvirtide costs for about \$25000 in United States. However, its expensive costs and inconvenience have made it only as a reserve. Fusion inhibitors can interfere with the process of adsorption by preventing the contact between human cell receptors and virus 'Spike protein. If we can find an appropriate Fusion inhibitor specifically targeting COVID-19, we can kill the virus at the very start of its proliferation process. The second method that I propose is based on the enzyme Reverse Transcriptase; reverse transcriptase can efficiently block the action of Integrase and thus prevents the replication of strands. Reverse Transcriptase has also been widely used in treating HIV. Similarly, perhaps we can develop Reverse Transcriptase in treating COVID.

Conclusion

Conclusively, most of the drugs against COVID-19 are on their developing process, so up to now there is no efficient drug. According to IHME's estimation, By October 1, 2020, cumulative

COVID-19 deaths could reach 179,106 deaths (with an estimated range of 159,497 to 213,715). In terms of the mean projection, this represents approximately 60,000 additional cumulative COVID-19 deaths until October 1, 2020. When the pandemic will end is still unknown[14].

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