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About A Lockdown: Are the Novel Etiological Therapies for the SARS-CoV-2 Effective?

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Abstract

Even though the humankind is familiar to disease outbreaks and pandemics, one of the few that has negatively impacted not only public health, but economy, finances among other vital government sectors is the COVID-19 caused by the SARS-CoV-2. These Viral family are common pathogens that can cause a variety of clinical entities, from respiratory symptoms to gastrointestinal ones ranging from mild to death threating cases. Many countries worldwide had already reported numerous index and imported cases and the death toll continues to trend up most dramatically in European and Asian countries. For this reason, one of the main strategies to control and contain the widespread dissemination of the virus are social isolation and even lockdown. In spite this measure, the number of cases continues to trend up with the lethality rate and the burden of the disease slowly increasing. Even though this disease presents a challenge, not only in diagnosis, but in specific etiological treatment, many researchers are developing many SARS-CoV-2 specific drugs and agents to decrease the alarming numbers of mortality and death toll. Given those treatments are novel, most of them have increasingly growing information regarding effectiveness and safety. In this article, we will try to compilate all the current novel therapies that are currently being developed for this on-growing pandemic.

Background

The first few cases of this novel Coronavirus appeared in early December 2019, in the city of Wuhan, China. This first poll of patients was composed by 44 new cases of an unknown entity pneumonia, among which some had a common history of contact with wild animal's trade in the Huanan Seafood Wholesale market. [1]. In January of this year, a working group organized by doctors, epidemiologists and other scientists discovered the causal agent, called 2019-nCoV [2]. As the days passed, the increase in new cases within the city and the appearance of cases outside of China began to attract the attention of the entire scientific community. Prevention measures began to be applied throughout the country, such as social isolation and the application of quarantine laws. Despite Chinese efforts, at the end of January, the first patient with a positive result of the 2019-nCoV virus was found in France. Few days later, new cases began to appear in various countries in Europe (Germany, Italy, Spain) [3,4] and America [5]. Following the trend that occurred in China, the virus spread geographically until on March 11, 2020, the WHO declared the COVID 19's pandemic (new name of the previously called virus 2019 - nCoV). At the time of writing this article, 417 966 cases have been confirmed worldwide, with a total of 18 615 deaths, with Italy, Spain and the United States being the new sources of infection [6].

Various studies have been carried out throughout these months and a lot of useful information has been discovered. It is known that Covid19 can affect patients of all ages, both sexes. It is compatible with cough, fever (> 37.5 C), myalgia, fatigue, rhinorrhea, nausea or vomiting, diarrhea or even respiratory distress that can lead to acute respiratory distress syndrome and to death [7,8]. Several studies have been realized to predict which of these patients going to present serious complications. It has been shown that advanced age (> 70 years), have comorbidities as hypertension, coronary heart disease or diabetes, high fever (> 39 C), initial tachypnea or dyspnea, accomplish qSOFA criteria (FR> 22, sensory disturbance, MAP <100), a high CURB65 score, leukocytosis or leukopenia, lymphocytosis or lymphopenia, thrombocytosis or thrombopenea, elevated LDH, ferritin or procalcitonin [9,10] predisposes to severe disease. The treatment of these severe manifestations is merely symptomatic and is based on support measures pending the immune response of the guest [11,12]. For the medical community, this situation is an emerging problem due to the few specific tools that are available for the Covid 19. For this reason, the

main scope of this article is to give a focused review of the pathophysiology of the SARS-CoV-2 and novel therapies being developed and used to treat this viral entity.

Virology and pathogenesis

Coronavirus are a widespread subfamily of enveloped, RNA single stranded positive viruses that can infect either humans or animals [13] being the most important reservoirs bats (Rhinolophus affinis) and Civets (Civettictis civetta) [14]. Inside this viral subfamily, four genera Alpha, Beta, Gamma and Delta exists; but the only ones that could potentially cause diseases in humans are the first two [15]. The Beta Coronavirus genera is the most feared one since it has strains, such as SARS-CoV, MERS-CoV and the new SARS-CoV-2; that can cause severe respiratory insufficiency and other clinical traits such as hepatic insufficiency and neurologic disturbances [16]. Upon complete genomic sequencing, the resemblance between the SARS-CoV-2 and its other genera counterparts are a least 95% [17] with its main differences in the mutation and adaptability rate, being the SARS-CoV-2 less dramatic [18] than the MERS-CoV and SARS-CoV. The genome of the SARS-CoV-2 encodes 2 polyproteins (ppa1a and pp1b), 16 non-structural proteins (NSP) translated from one of the first open reading frames (ORF 1a/1b) and 4 structural proteins that elicits attachment, transmembrane transport and interferes with host immune response [15]. The first of these structural proteins is the Spike (S) Glycoprotein which elicits the entry to the Type II pneumocyte through the ACE2 receptor. After the interaction of the receptor with the S1 subunit, the envelope of the virus fuses with the host cell through the S2 subunit [18,19]; thus, releasing the RNA to the cytoplasm and promoting the formation of a double membrane vesicle called the Replication-Transcription Complex (RCT) [20]. From this start-point, more RNA is synthetized for further encoding of accessory and other structural proteins [15,19]. The cytoplasmatic RNA concentration spike activates the sensors RIG-I and MDA 5, that starts innate immune response [21]. These sensors ends-up in the phosphorylation of IRF-3 and starts the Interferon-I (IFN-I) transcription [15,21]. Another innate immune response is the cytoplasmic increase of catalyse 2,5-oligoadenylate (2'-5'OA) that serves as a second messenger for the activation of RNAse L which serve as cleavage for viral st-RNA [22, 23]. At this point, the Matrix (M) protein of the SARS-CoV-2 directly induces the suppression of the IFN-I transcription along with the highly sensitive to ds-RNA ORF4a which transcripts nsp-1 that cleaves host's mRNA. [18,21] Other mechanism of immune suppression is the activation of ORF4b which encodes NS2a that serves as phosphodiesterase for the 2'-5' OA; completely shutting down the host cell immune response [23]. Finally, once the host cell is completely infected, the SARS-CoV-2 starts a cytokine storm by the upregulation of IL-1-β driven by the ORF3a and Envelope (E) structural protein [18,23]; overall causing cell death and cytopathy through pyroptosis, necrosis and apoptosis. The membrane ruptures further sheds more viral particles, thus promoting the interaction and infection of other host cells.

Vaccines: Are we there yet?

Multiple advances are being developed to find an efficient and safe vaccine to generate immunity in the host. Most of the advances are based on the target surface - exposed spike (S protein) glycoprotein as the greatest generator of cellular and humoral immune response [24,25]. Various strategies have been carried out, such as using the entire S protein or only cell-anchored protein regions (S1 subunit RBD region) and expressing it in virus-like particles, DNA or viral vectors to generate immunogenicity [26,27]. This technique intends to obtain antibodies that inhibit viral-cellular binding and viral uncoating. One of the actions is to use the C-terminal region of the RBD in porcine Delta Coronavirus [28]. It is planned to use viral vectors (Adenovirus, rabies virus) to achieve immunity against MERS CoV, due to the immunogenic similarity of SARV-CoV-2 and MERS CoV [29]. Despite the genetic similarity between the SARV-CoV and the SARV-CoV-2, when comparing the protein S of both viruses, it was discovered that in the immunogenic region of the first (S1 subunit) it had multiple variable residues [30], so it could be that if a SAR-CoV vaccine is injected in the host, a humoral response against SARV-CoV-2 will not develop. Other viral particles that are planned to generate an immune response are viral epitopes that interact with cytotoxic T lymphocytes [31]. By March 20, 2 phase 1 vaccines [32] are available by the WHO. The first one was done in Beijing, China using a Non-Replicating Viral Vector (adenovirus). Said vaccine is planned to be inoculated in healthy young people between 18 and 60 years old, currently in the participant recruitment phase. The first results of phase 1 are expected to come out in approximately 6 months [33]. The second, carried out by the National Institute of Allergy and Infectious Diseases in the United States, plans to use an mRNA that encodes protein S in its entirety. There are 45 young healthy subjects who will receive the vaccine for the first time on day 1 and for the second time on day 29 and follow them for 12 months to see safety, reactogenicity and immunogenicity. Currently the study has already started, and the first results will be released in June [34]. Additionally, 42 vaccines are in preclinical stage.

Hydroxychloroquine: A multi-use drug?

Hydroxychloroquine is a drug mainly used to treat malaria and various rheumatic diseases. Has multiple mechanism of action, the principal is to increase the liposomal pH in the antigen presenting cells (ACP), causing a decrease in the TLR signalling of the dendritic cells, decreasing the presentation of antigens to the T cells with anti-inflammatory effects [35]. It also interferes with the union of various viruses with the cell, altering the synthesis of sialic acid in the binding ligands, inhibiting virus-cell union [36]. It is part of the WHO essential drugs list, is used worldwide due to the efficacy, safety and economic accessibility that has [37]. In vitro effectiveness has been seen in the treatment of various infectious diseases such as HVB, HVA, polio, influenza virus A and B, and finally SARS-CoV-1, where it shows to inhibit viral replication in lung cells [38, 39]. On February 2020, a research letter published an in vitro experiment evaluating the efficacy of

hydroxychloroquine in Vero E6 cells infected with SARS-CoV-2, demonstrating the decrease of viral replication at an effective concentration of 6.90 UM [40]. These in vitro studies began to be replicated in various cities in Asia and Europe, demonstrating the efficacy and safety against Covid19 at doses of approximately 200 mg-500mg twice a day for 10 days [41,42]. Various clinical trials are currently underway to see if such effectiveness and safety can also be demonstrated in vitro. These studies, still in the recruitment phase of participants, will use varying doses between 0.1g BID and 1g every 2 days [43]. Despite all good pre-clinical evidence, the WHO and CDC have not established specific recommendations for the use of hydroxychloroquine due to lack of evidence in randomized clinical studies in real patients.

Remdesevir: A faithful copy-cat?

In the 2016, after the Ebola Virus outbreak, a monophosphoromidate pro-drug called GS-5734 was developed by Gilead Sciences for the treatment of this disease [44]. At that time, the compound was used in multiple murine and non-human primate models [45,46] and showed good distribution to peripheral blood mononuclear cells, turning to its active form after 2 hours of infusion with an acceptable intracellular half-life of 14 hrs [47]. It also showed relatively low half-maximum effective concentrations (EC50) in tissue cultures [47]. Afterwards, one of the first clinical experiences with the drug was a compassionate-use treatment of a British nurse suffering of Meningoencephalitis caused by Ebola Virus that resulted in the resolution of the entity along with any complications [48]. This drug, renamed as Remdesevir (RDV), also exhibited antiviral activity against Marburg Virus [46], Members of the Paramyxoviridae family and Pneumoviridae [49]; demonstrating the broad-spectrum antiviral activity. Regarding the use of RDV in the SARS-CoV-2 outbreak, reports in primary human airway epithelial cell cultures showed inhibition of the virus along with less lung cytopathy [47]. The mechanism proposed for these effects is that the triphosphate form of this nucleotide analogue functions as a competitive inhibitor of the divergent RNA-dependent RNA polymerase composed of nsp7, nsp8 and nsp12 [50]. This causes arrested RNA synthesis with a delayed chain termination due to the exchange of normal viral ATP to a stop signalling in i+3 and i+4 positions [50]. Also, probable viral resistance to RDV was tested with a model of a Beta coronavirus called Murine Hepatitis virus (MHV) and showed that two amino acid substitutions (F467L and V553L) elicited an increase in intracellular EC50 [51] due to the exonuclease effect of nsp14 that these mutations promoted [52]. In spite this trait, the viral fitness of the mutant strains is decreased and are mostly outcompeted by Wild-Type strains of the MHV model [46, 50] and probably confers a protective motif for the RDV against future resistance. Using this knowledge, RDV was used in rhesus macaque models to test the efficacy of prophylactic and therapeutic models [53] and elicited promising results such as preventing clinical disease, inhibited replication in tissues and improved pulmonary function [53]. This preliminary data was used for the treatment of a patient with COVID-19 in Washington, DC that resulted in improved clinical condition without any adverse effect [54]. In this matter, two phase 3 RCT are being performed in China to evaluate the efficacy and safety of parenteral RDV in regimens that resulted with positive outcomes in Ebola virus diseases treatment [55]. We as a community must wait for the results of these promising RCT's and decide if the RDV would be beneficial as treatment.

Lopinavir/Ritonavir: Similarities between HIV and SARS-CoV-2?

In mid-2003, in south China, the first severe and transmissible epidemic appeared, SARS epidemic [56]. Due to the need to find an efficient therapy, in 2004 a study was carried out with Lopinavir, a type 1 aspartate protease inhibitor and Ritonavir, a cytochrome p450 inhibitor, two world-known drugs whose combination is used to fight the Immunodeficiency Virus human type 1 (HIV-1) disease. It demonstrated a decrease in the SARS-CoV viral load and a lower adverse clinical outcome [57]. However, due to methodological flaws, it could not be verified if this result could happen in the general population. Additionally, various case reports mentions the use of lopinavir/ritonavir has a positive outcome with MERS-CoV [58], but cause lack of human clinical trials, this cannot be demonstrated. Considering these antecedents, a recently published study was made using lopinavir/ritonavir to treat the new SARS-CoV-2 epidemic to demonstrate its efficacy and safety [59]. The result of the study showed that the use of Lopinavir / Ritonavir was not found to be associated with a decrease in viral load or clinical improvement or mortality in a population with severe manifestations, however, it found that in clinically stable patients, the time to clinical improvement improved despite the adverse effects (nausea, vomiting, diarrhea, abdominal discomfort, leukopenia, lymphopenia) [59]. More evidence is needed to recommend the use of this medicine against COVID infection19.

Interferon: A helpful coadjutant?

As explained before in this article, IFN is one of the first innate immune response elicited by the cell host to prevent further cell infection. The main mechanism of action is to modulate various expression of immune genes that leads to activation, growth and differentiation of NK cells and T cells among others [60]. Physicians have plenty expertise using this molecule as a broad-spectrum antiviral agent, especially in Hepatitis B and C treatment [61]. Regarding the use in the Beta Coronavirus subgenera, in previous years of the outbreaks of SARS-CoV and MERS, various non-human primate models showed that coadjutant treatment with Ribavirin [62] and LPV/r [63] did not developed respiratory insufficiency along with no lung histopathologic abnormalities [62,63]. In addition to this serum, urine and BAL viral loads were lower in the treated group with these drugs [62,63]. In Spite these promising results, specimens in the LPV/r study died unexpectedly and developed subtle weight loss over the course of the treatment [63]. With these data, in 2014 Omrani, et al performed a retrospective case-control study comparing

20 patients being treated with Ribavirin + IFN alfa-2a and 24 patients without it [64]. The results were positive for 14-day survival benefit compared to the control group along with significant decrease in neutrophil levels [64]. One of the main adverse effects in the treatment group was decreased of a mean of 4.32 gr/dL in haemoglobin levels [64]. Regarding the use of IFN in SARS-CoV-2, some guidelines [65] use alpha interferon atomization as an inhaled drug along with LPV/r as weak recommendation with low levels of evidence. In order to reduce the gap knowledge and given that the MERS and SARS-CoV-2 share a close resemblance, the MIRACLE trial is being performed in China in order to evaluate the efficacy and safety of this combination [66] with the primary outcome of 90-day mortality along with various secondary outcomes such as ICU mortality, In-hospital Mortality, Vasopressor-free days among other variables [66]. By the time of this article, the trail is still enrolling patients and the main data result is still not available.

Conclusions

Even though the novel strain of Beta Coronavirus continues to increase the number of new cases and mortality rate with dramatic death toll data, especially in Italy, many drugs with uses on other diseases are currently being tested for this new strain such as LPV/r and Hydroxychloroquine. Even drugs developed at first for other viral entities such as IFN and RDV are showing promising data on human and non-human models for the treatment of this novel strain. In addition to this, vaccines using structural viral proteins such as S protein are being developed given that it exerts immunogenicity. To wrap up, in spite the high virulence and mortality rates that we are experiencing with this pandemic, all these treatments shed a light at the end of the tunnel to solve this challenge nature has given to us. The only thing we must do is continue with proper patient care and wait for the data of the many RCT´s being performed with these agents.

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