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Opinion

A SARS-CoV-2 Prophylactic and Treatment; A Counter Argument Against The Sole Use of Chloroquine

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Abstract

A better knowledge of the SARS-CoV-2 virus and its underlying pathobiology is accumulating every day. Of huge importance now is to provide a fast, cost effective, safe, and immediately available pharmaceutical solution to curb the rapid global spread of SARS-CoV-2. This Opinion discusses the demands for such an ideal drug and taking into account an aspect of viral mechanisms of infection. An effective prophylactic medication to prevent viral entry has to contain, at least, either a TMPRSS2 inhibitor or a competitive virus ACE2 binding inhibitor. Using bromhexine at a dosage that selectively inhibits TMPRSS2 and, in so doing, inhibits TMPRSS2-specific viral entry is likely to be effective against SARS-CoV-2. We propose the use of bromhexine as a prophylactic and treatment. We encourage the scientific community to assess bromhexine clinically as a prophylactic and curative treatment. If proven to be effective, this would allow a rapid, accessible and cost-effective application worldwide.

Keywords: SARS-CoV-2; COVID-19; Coronavirus; Prophylactic; Treatment; Anti-viral drugs; Drug combinations; Bromhexine

Introduction

As the world witnesses the alarming levels of spread and severity of atypical pneumonia COVID-19, strategies to combat this outbreak are in dire need. The first sequence of SARS-CoV-2 was published online one day after its confirmation on behalf of Zhang and colleagues [1]. SARS-CoV-2 sequences isolated from all over the world have now been deposited in gene banks [2,3]. Sharing more genome sequences of the newly emerging SARS-CoV-2 allows analysis of this new coronavirus (CoV), improving phylogenetic analysis and, most important, recognizing mutations between differing strains. Identifying the closest viral relatives of SARS-CoV-2 is greatly assisting studies of viral function. Ultimately, this gives rise to the understanding of what is unique and what is conserved in this new SARS-CoV-2 virus; be it structure, its host cell attachment and entry, or replication, making it possible to identify treatment targets. Currently, the treatment is mainly symptomatic and supportive care. Tremendous efforts have been undertaken and large amounts of money have been invested in vaccine development against influenza-type viruses. There are approximately 40 companies in advanced stages of vaccine development [4]. Disadvantages with cutting-edge vaccines are that they take months to years to develop and to approve, and they become obsolete if the virus evolves. There are already a number of reviews on potential treatment strategies against COVID-19 [5-7]. Drug repurposing is an attractive alternative drug discovery strategy in this time of urgency.

Proposed Treatment Strategy Against COVID-19

The first step in CoV infection is the interaction of host cells with the viral envelope Spike (S) glycoprotein.SARS-CoV-2 employs two routes for host cell entry, which are dependent on the localization of the proteases required for activation of the S protein [8]. Binding of SARS-CoV-2 to the cellular receptor, angiotensin converting enzyme 2 (ACE2), can result in uptake of virions into endosomes, where the S protein is activated by the pH-dependent cysteine protease cathepsin B and L (cathepsin B/L) [9-11]. Alternatively, the spike protein can be activated by the serine protease TMPRSS2, resulting in fusion of the viral membrane with the plasma membrane [12]. Seeing as the S protein has pivotal roles in viral infection [13] we propose interfering with the S protein activation and hence viral pathogenesis.

Recently, publications on COVID-19 have brought attention to the possible benefit of repurposing the drug chloroquine in the treatment of patients infected by SARS-CoV-2 [14,15]. Chloroquine (N4-(7-Chloro-4-quinolinyl)-N1,N1-diethyl-1,4-pentanediamine), an FDA-approved drug [16], has been used to treat malaria and amebiasis for many years [17], as well as autoimmune diseases. Viral fusion and release of the genetic components is highly dependent on the endosomal pathway and particularly pH. Chloroquine can affect virus infection in many ways. Of particular importance is that Chloroquine is known to block virus infection by increasing endosomal pH required for virus/endosome fusion [18] and release of viral RNA into the cytosol. Past research on chloroquine has shown in vitro activity against many different viruses, but no benefit in animal models [19]. Chloroquine in almost all animal models of different viral infections only partially worked or didn't work [20-22]. Treatment with chloroquine did not prevent influenza infection in a randomized, double-blind, placebo-controlled clinical trial.23 Conversely, it worked very well in vitro [24-26]. This could indicate that the main mechanism of action of chloroquine, in vivo, is via interference with the unspecific endosomal pathway. The extracellular concentration of the orally applied chloroquine, especially in lung tissue, in vivo, may not be high enough to inhibit virus binding via glycosylation of the binding pocket [18]. After the viral infection has spread in the body and due to the incredibly high viral loads, the unspecific pathway is mainly used for further virus replication. This may explain the recent success reported with chloroquine to assist in the curing of the virus. Whether chloroquine can treat COVID-19 alone and also work as a prophylactic is doubtful. This needs to be further investigated before masses of people start to take this relatively toxic drug as a preventive measure.

Inhibition of the serine protease, TMPRSS2, activity is an excellent target for antiviral intervention. Hoffman et al. [27] suggested that TMPRSS2 could be a potential therapeutic target for COVID-19 since entry of the virus into cells was reduced by camostat mesilate, a non-selective TMPRSS2 inhibitor. Non-selective inhibitors have greater, more severe side effects than selective inhibitors and currently camostat mesilate is only approved for treatment of chronic pancreatitis [28,29] in Japan. Unfortunately, the drug is costly and won't be available to treat large-scale patient numbers.

TMPRSS2 is expressed highly in localized high-grade prostate cancers and in the majority of human prostate cancer metastases. Lucas et al. [30] showed a decrease in the frequency of metastases and a slowdown of the spread of metastases in mice with prostate cancer by using TMPRSS2 inhibitors. In particular, they identified bromhexine, an FDA approved ingredient [30] in mucolytic cough suppressants, as a potential TMPRSS2 inhibitor for their application. Bromhexine is orally readily bio-available.. Endonasal application is also a good alternative option. Bromhexine is an over-the-counter (OTC) drug [31] that is affordable with proven safety. Typically bromide compounds, especially aromatic bromide compounds, show a relatively high binding affinity for serine-containing peptide sequences, proteins and enzymes [30,32] Lucas et al. [30] show that this effect is due to a selective inhibition of TMPRSS2 by bromhexine. The available data suggests further that ambroxol, a metabolite of bromhexine, is a potent inducer of surfactant synthesis in AT2 cells [33-35]. Its lung protective properties have been discussed in infants and severely ill adult patients as well as the potential as an adjuvant in anti-infective therapy [34]. Thus, bromhexine also provides indirect protective effects. Laporte and colleague, Naesens [36], reported that bromhexine did not show any significant cell entry or replication inhibition effect in vitro in Influenza viruses. However, the authors showed that Influenza viruses utilize, contrary to SARS-CoV-2, a different extracellular host protease for priming. Thus, these results are not representative for SARS-CoV-2 [36].

In already infected individuals we believe it is essential to combine the lesser toxic chloroquine-derivate, hydroxyl chloroquine, with a TMPRSS2 inhibitor, like bromhexine, to block complete entry of the virus into host cells. In the case of prophylaxis the inhibition of the TMPRSS2 is essential [27] and the non-specific endosomal entry is negligible. An effective prophylactic medication to prevent viral entry has to contain, at least, either a TMPRSS2 inhibitor or a competitive virus ACE2 binding inhibitor. This will prevent further spreading of the virus through the host's body.

Conclusion

A prophylaxis strategy and a suitable treatment for the emerging SARS-CoV-2 is crucial for reducing the mortality and morbidity of this disease but developing and obtaining regulatory approval for new drugs can take years and is discordant with the urgent need for a therapy. Drug repurposing is an attractive alternative drug discovery strategy because there is the advantage of ease of

access, decreased cost of development (as they have established manufacturing arrangements), and the possibility to provide a wide array of options for combination studies. The background pharmacological knowledge available for such compounds may also reduce concerns regarding adverse effects in patients as they have gone through rigorous safety and risk testing and are already approved as safe for human use. Using bromhexine at a dosage that selectively inhibits TMPRSS2 and, in so doing, inhibits TMPRSS2-specific viral entry is likely to be effective against SARS-CoV-2. We propose the use of bromhexine as a prophylactic and treatment. Furthermore, a combination with hydroxyl chloroquine, that is (amongst other functions) an effective endosomal protease inhibitor, inhibiting cathepsin B/L, could be a favorable combination for the treatment of moderate to severe COVID-19 cases. This combination would block virus-host cell entry completely by blocking the specific receptor mediated entry (via bromhexine) and endocytotic virus entry (via hydroxychloroquine sulfate). We can only encourage the scientific community to test bromhexine and the combination with hydroxychloroquine and to follow our recommended approach in order to also identify further ideal repurposing candidates according to the herein proposed criteria.

Conflict of Interests

The authors have declared that no conflicts of interest exist.

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