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Azithromycin and ambroxol as potential pharmacotherapy for SARS-CoV-2

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ABSTRACT

Knowing the ability of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to bind to the angiotensin-converting enzyme 2 (ACE2) receptor and to enter cells via endocytosis paved the way for repositioning of old drugs as potential treatment of COVID-19, the disease caused by SARS-CoV-2 infection. This paper highlights the potential of azithromycin and ambroxol to treat COVID-19. Azithromycin and ambroxol share lysosomotropic characteristics, i.e. they penetrate and accumulate inside the late endosomes and lysosomes and may possibly interfere with multiplication of the virus inside cells. In addition, both of these drugs have anti-inflammatory effects. Ambroxol has a proven antiviral effect and a unique stimulatory action on the secretion of surfactant by alveolar type II cells, the main target of SARS-CoV-2. Surfactant may form a fundamental defence mechanism against the virus. Involvement of nasal epithelial cells in SARS-CoV-2 entry suggested advantageous use of inhaled drug delivery of these two drugs over the use of systemic administration. Inhaled drug delivery could aid in targeting the drug to the exact site of action with little or no side effects. To conclude, administration of these two drugs using a special drug delivery system provides two kinds of drug targeting: (i) tissue targeting through using an inhaled drug delivery system to achieve high drug concentrations at the respiratory epithelial tissue that overexpress the ACE2 receptor for virus binding; and (ii) cellular targeting of the virus in the acidic vesicles (late endosomes and lysosomes), which represent the fate of endocytic viruses.

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1. Introduction

Coronaviruses (CoVs) belong to the family Coronaviridae that consists of four genera, α-CoVs, β-CoVs, γ-CoVs and δ-CoVs. The viruses responsible for the outbreak in China in 2003, known as severe acute respiratory syndrome coronavirus (SARS-CoV), as well as the second outbreak caused by Middle East respiratory syndrome coronavirus (MERS-CoV) that caused severe acute respiratory disease in the Middle East in 2012 are β-CoVs [1].

In the last week of 2019, a novel coronavirus, now know as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused an outbreak in Wuhan, China. It is believed that it started in a seafood market and then spread very rapidly through Wuhan City and China. The disease caused by this virus has been named coronavirus disease 2019 (COVID-19). SARS-CoV-2 spread very quickly in China and then to many other countries, causing a life-threatening global pandemic.

Clinical features of COVID-19 include fever, cough and pneumonia leading to death in certain cases [2]. Reported deaths in Europe have increased in a way that every effort should be made to work together to fight the disease. On 12 March 2020, the World Health Organization (WHO) announced COVID-19 as a worldwide pandemic. To date, millions of people worldwide have been infected with SARS-CoV-2 and hundreds of thousands have died due to COVID-19.

Many pharmacological and non-pharmacological treatments have been tried and a total of 238 registered clinical trials were conducted before the end of February 2020, some of them including repositioning of old drugs (https://www.cebm.net/oxford-covid-19/covid-19-registered-trials-and-analysis/). Several reports suggested lysosomotropic agents such as chloroquine and hydroxychloroquine as potential pharmacological treatments. Indeed, hydroxychloroquine has been used in China and other countries and it has been shown that hydroxychloroquine is effective against SARS-CoV-2 in vitro [3].

2. Lysosomotropic agents

Lysosomotropic agents are cationic (basic) drugs that are protonated and become trapped inside the late endosomes or lysosomes. This process is known as lysosomal trapping where any

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lipophilic (logP > 1) or amphiphilic drug with ionisable amines (pKa > 6) can accumulate in lysosomes. This process contributes to pre-systemic extraction of drugs by lysosome-rich organs such as the liver and lungs [4].

The pH gradient between the neutral cytosol (pH 7.0–7.2) and the acidic lysosome (pH 4.5–5) is the major driving force delivering lysosomotropic drugs into the lysosomes via passive diffusion [5]. This is followed by a protonation step of basic groups in the acidic environment of lysosomes or any acidic counterparts such as late endosomes, which converts the neutral molecule into a protonated molecule that cannot diffuse out of the lipid bilayer. Lysosomal trapping during cellular drug uptake leads to extremely high drug concentrations within the lysosome in comparison with the cytosol [6].

2.1. Endocytosis as a mode of cellular uptake of coronaviruses

Briefly, endocytosis acts as the main mechanism for CoV uptake into various cell types. However, of the different endocytosis routes, clathrin-mediated endocytosis and cathepsin-mediated S protein cleavage are the two critical endocytic pathways for viral entrance and infection of SARS-CoV-2 [7].

Following endocytosis, internalised cargoes enter the early endosome where they are either recovered for recycling to the plasma membrane or are delivered to the late endosome that fuses with the lysosome for subsequent degradation [8]. The main functions of the endocytic pathways are for retrieval and recycling of internalised cargo proteins. These processes play key roles in the pathophysiology of some human diseases such as viral infections and neurodegenerative diseases, e.g. Parkinson's disease [9,10].

Possible involvement of the endocytic pathways as a cellular uptake mechanism of the newly emerging SARS-CoV-2 has been reported [11]. It is now known that SARS-CoV-2 can enter host cells utilising the same receptor as SARS-CoV, namely angiotensin-converting enzyme 2 (ACE2) [12]. ACE2 receptors are present in the epithelium of the human lungs and small intestine [13]. However, very recently, ACE2 receptors were also identified in the epithelial cells of the nose and upper respiratory tract [14]. Another recent study showed that although SARS-CoV-2 binds to the same ACE2 receptor as SARS-CoV, binding in the case of SARS-CoV-2 is with higher affinity than for SARS-CoV [15]. Knowing that SARS-CoV-2 can enter cells through endocytosis may explain the effectiveness of some lysosomotropic agents such as chloroquine and hydroxychloroquine [16,17].

2.2. The importance of autophagy

Autophagy is a highly conserved process in which intracellular components such as damaged organelles and protein aggregates are sequestered into a double-membrane vesicle called the autophagosome. The autophagosome then fuses with the lysosome to form the autolysosome for degradation and ultimate recycling of the resulting macromolecules [18,19]. Autophagy plays a vital role as a host defence mechanism against viral infections [20]. In the case of MERS-CoV, some viral proteins lead to the induction of incomplete autophagy, however further work is needed to confirm the role of autophagy in the antiviral immune response [21,22].

3. Repositioning or repurposing of two old drugs

Owing to the high risk and rapid spread of COVID-19, there is an urgent need for an approved drug in order to save the lives of millions of people. Repositioning of old drugs is a good approach to introduce drugs in a short time without waiting for lengthy preclinical and clinical studies. This article suggests the repositioning of two drugs, namely azithromycin and ambroxol.

3.1. Azithromycin

Azithromycin, which is a member of the macrolide class of antibiotics used for infectious diseases, was approved by the US Food and Drug Administration (FDA) in 2002. Two characteristics of azithromycin proposed it as a potential therapy for SARS-CoV-2 infection.

First, azithromycin has favourable physicochemical properties. It is a dicationic drug with high lipid solubility (logP = 3.03) that can accumulate in the acidic cellular compartment, meaning it can be considered a lysosomotropic agent. Indeed, azithromycin has distinctive effects on endocytosis; it not only interferes with the acidification process but also helps in dissecting various steps of the endocytic apparatus, including fusogenicity with lysosomes [23].

Second, azithromycin has an antiproliferative and autophagic effect on airway smooth muscle [24]. In addition, azithromycin inhibits arachidonic acid release and prostaglandin E2 synthesis, with consequent impairment of lysosome function that may lead to an anti-inflammatory effect [25].

An inhaled formulation of azithromycin may be advantageous. It can be prepared as a dry powder for inhalation using spray-drying technology with the aid of a suitable diluent. A dry powder formulation of azithromycin was successfully achieved with good inhaler characteristics suitable for inhalation delivery [26].

Very recently, supportive evidence came from French studies about the potential benefit of azithromycin in treating COVID-19. These studies indicated that using azithromycin in combination with hydroxychloroquine resulted in a beneficial effect in the treatment of COVID-19 at an early stage and might reduce the contagiousness of the disease [27,28]. However, these studies were observational studies conducted on small numbers of patients and there are arguments about the safety of such a combination. Nevertheless, there is a need for well-designed controlled studies to confirm the assumed role of azithromycin.

3.2. Ambroxol

Ambroxol is a mucolytic agent used in the treatment of respiratory diseases. Ambroxol is a basic (pKa = 9.01) cationic drug with lipophilic properties (logP = 2.9), enabling it to act as a lysosomotropic agent. In addition, ambroxol exhibits a novel mechanism by accumulating in lamellar bodies and acting as a lysosomal secretagogue. This may also play a role in its use for lysosomal storage diseases [29]. Moreover, ambroxol can induce autophagy, which may have a role in fighting SARS-CoV-2 [30].

The main effect of ambroxol is on mucus regulation, however a wide range of pharmacological effects of ambroxol have been confirmed, including anti-inflammatory, reduction of arachidonic acid metabolites and pro-inflammatory cytokines, and antioxidant properties. In addition, ambroxol aids in the enhancement of local defence molecules involved in respiratory viral replication [31].

It has been reported that SARS-CoV-2 binds to alveolar type 2 (AT2) epithelial cells where ACE2 receptors are highly expressed [32]. AT2 cells are responsible for the production of surfactant. One mechanism of action of ambroxol is to act specifically on AT2 cells and to stimulate the release of surfactant, which aids in preventing influenza virus multiplication and in maintaining alveolar function and preventing alveolar collapse [33].

The antiviral effect of ambroxol may be due to upregulation of natural inhibitors of proteases that represents a potential therapeutic approach to suppress viral airway replication. A study conducted on rats showed that treatment of animals with ambroxol prior to infection with influenza A virus led to a substantial suppression of virus multiplication as well as improved survival [33].

Despite the presence of registered clinical trials on the prodrug bromhexine, the precursor of ambroxol (https://clinicaltrials.gov/ct2/show/NCT04355026), there is no registered clinical trial on ambroxol in COVID-19. Interestingly, one Chinese study mentioned the use of ambroxol as symptomatic treatment of cough in children with COVID-19 [34]. In addition, there are three case reports mentioning the use of ambroxol as symptomatic treatment for relief of phlegm [35,36].

As mentioned previously, there is evidence that SARS-CoV-2 may invade host cells through binding to ACE2 receptors present in the nasal cavity. Accordingly, inhaler administration of ambroxol may represent an efficient delivery method of ambroxol to the target site with fewer systemic side effects. Notably, ambroxol inhalation has been approved as an efficient and convenient therapy for other respiratory diseases [37] and is available in some countries as an ampule for inhalation.

4. Conclusion

Both azithromycin and ambroxol have lysosomotropic properties, allowing these two drugs to block virion infectivity. In addition, they have anti-inflammatory effects, while ambroxol has an additional proven antiviral effect and a surfactant stimulatory effect that may contribute in the innate immune mechanism against the virus.

The point highlighted here is the necessity of exploiting recent knowledge about the involvement of nasal epithelial cells in SARS-CoV-2 entry as well as knowledge of the involvement of endocytosis as a cellular uptake mechanism for SARS-CoV-2. This work suggests the repositioning of two old drugs, namely azithromycin and ambroxol, with the advantageous use of inhaler drug delivery over the use of systemic administration. Further investigations are required to confirm the effectiveness of these two drugs. It is worth mentioning here that both of these candidates (azithromycin and ambroxol) lack the serious side effects encountered with other lysosomotropic agents such as chloroquine and hydroxychloroquine.

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