Potential value of pharmacological agents acting on toll-like receptor (TLR) 7 and/or TLR8 in COVID-19

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ABSTRACT

The COVID-19 pandemic is an ongoing global pandemic of coronavirus disease 2019 as an atypical type of viral pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Many potential pharmacotherapies are currently being investigated against this disease. This article points to and justifies the importance of investigating the potential therapeutic value of pharmacological agents acting on Toll-like Receptor (TLR) 7 and/or TLR8 as double-edged swords combating COVID-19. Induction of TLR7 and/or TLR8 may be investigated as a strategy to stimulate immunity and may be added to anti-COVID19 vaccines to cope with their current viral escape challenge. TLR7 stimulation may not only help viral clearance through Th1 antiviral responses but may also provide beneficial broncho- and vaso-dilatory, as well as, anti-inflammatory effects. Pharmacological compounds acting as TLR7 and/or TLR8 agonists may be of value if used by frontline healthcare workers with comorbidities who demonstrate mild symptoms of the disease. On the other hand, TLR7 and/or TLR8 antagonists may be used in combination with immune-modulatory/anti-inflammatory drugs in severe cases of the disease, with potential synergistic effects that could also help in reducing the doses of such therapies, and consequently their adverse effects.

1. Introduction

Toll-like receptors (TLRs) are one class of receptors that play a key role in the recognition of viral particles, activation of the innate immune system, and consequently developing better drugs for COVID-19 (Khanmohammadi and Rezaei, 2021). In humans, TLRs have ten family members some of which are located in the cell membrane, and the others are situated in endosomes, such as TLR3, TLR7, TLR8, and TLR9 (Lester and Li, 2014). As pathogen recognition receptors (PRRs), their role in the pathogenesis of pneumonia should be stressed since alveolar macrophages express TLRs and are among the first cells to sense and respond to pathogens that enter the lungs (Quinton and Mizgerd, 2015; Steiner et al., 2017). TLRs were specifically reported to play important role in the induction of immune responses which lead to intracellular bacterium Mycoplasma pneumoniae eradication (Naghib et al., 2018). In addition, TLR signaling is known to be involved in the inflammatory response of the lungs and there has been an association between mortality in elderly patients with severe pneumonia and decreased TLR2 and TLR4 expression (Tang et al., 2014). Worth noting is that we previously pointed to and justified the importance of investigating the potential pharmacotherapies for COVID-19 “pneumonia”, rather than the broad category of COVID-19 disease (Khalifa and Ghoneim, 2020). TLRs may also have an important role in COVID pneumonia since viral particles could induce coagulation disorders through TLR3 activation (Biswas and Khan, 2020). RNA viruses in general, including SARS-CoV-2 causing COVID, invade the innate immune system leading to infection, through TLRs 3, 7, and 8 (Angelopoulou et al., 2020). TLR7 and TLR8 are of special importance since they are phylogenetically and structurally very similar and both can recognize GU-rich sequences in single-stranded RNAs from viruses (Forsbach et al., 2011). TLR7/8 are also involved in a plethora of intracellular signaling cascades which culminate in the gene expression of pro-inflammatory cytokines and chemokines (Parker et al., 2007). In addition, TLR7/8 is coupled to the adaptor protein MyD88 which activates downstream NF-κB driven genes (Kawai and Akira, 2007). It was also revealed that women were affected by COVID-19 less than men and it was suggested that this may be through a different
expression of TLRs, especially TLR7 and TLR8 (Conti and Younes, 2020). This was further explained by the levels of activation of the immune cells that are higher in women than in men, and it is correlated with the trigger of TLR7 that is higher in women than in men and its biallelic expression leads to higher immune responses and increases the resistance to viral infections. In addition, the X chromosome influences the immune system by acting on TLR8, among other proteins, which can be over-expressed in women, and influence the response to viral infections. Moreover, a higher frequency of rare TLR7 variants was reported in younger (~60 years) males with life-threatening COVID-19 than in a control group with asymptomatic or oligosymptomatic infection (Fallerini et al., 2021). Such deleterious TLR7 variants were suggested to account for up to 4% of severe disease in male subjects and these young males with TLR7 loss-of-function variants and severe COVID-19 represented a subset of male patients contributing to disease susceptibility in up to 2% of severe COVID-19.

1.1. TLR7/8 agonists

Antiviral effects of TLR7 agonists have been reported against human papillomavirus (HPV), herpes simplex virus (HSV), human immunodeficiency viruses (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) (Biffen et al., 2012; EnosiTuipulotu et al., 2018; Pockros et al., 2007a). It was also suggested that the available agonists against TLR7 may prevent the onset of severe COVID-19 in symptomatic patients and synergize with active anti-viral therapy (Onofrio et al., 2020). It was also hypothesized that remdesivir may synergize with TLR7 agonists and that the signs of efficacy of TLR7 agonists in combination with antiviral therapy may be captured by an early drop in viral load. In addition, we hypothesized earlier that the inhibition of TLR7 receptor function, by hydroxychloroquine may partially explain the ineffectiveness of this medication as an individual intervention in mild COVID since TLR7 function is critical for the activation of an appropriate innate immune response against ssRNA viruses that is utterly needed early in the course of the disease (Khalfa and Choneim, 2021). Imiquimod; as an immune-stimulator that activates TLR7, was also suggested to be used to enhance innate and adaptive immunity (Angelopoulou et al., 2020). As a potential pharmacotherapy for COVID-19, TLR7 stimulation may not only help viral clearance through Th1 antiviral responses (Medzhитов, 2001; Akira and Takeda, 2004) but may also provide bronchodilating and anti-inflammatory effects because these receptors are expressed in the bronchial epithelial cells and airway smooth muscle where the initiation of a Th1 immune response activation of TLR7 rapidly relaxes airway smooth muscle via production of NO (Lebold et al., 2016; Ekman et al., 2011). The produced NO may also selectively dilate pulmonary arteries which allow for more blood flow to open alveoli for gas exchange which is crucially needed in COVID-19 pneumonia patients. TLR8 has been also reported to recognize ssRNA viruses such as Influenza, HIV, HCV, Sendai, and Coxsackie B viruses, and the receptor binding to the viral RNA recruits MyD88 and leads to activation of the transcription factor NF-kB and hence an antiviral response (Heil et al., 2004; Zhang et al., 2016). Therefore, pharmacological compounds acting as TLR7 and/or TLR8 agonists such as imidazoquinoline, thiagozinolone, or benzazepine analogs may be of value if administered in combination with immune-modulatory/anti-inflammatory drugs. Induction of TLR7 and/or TLR8 stimulation may be investigated as a strategy to stimulate the innate immune system against ssRNA viruses, and consequently developing better drugs for COVID-19 (Khannanommad and Rezaei, 2021; Medzhитов, 2001; Akira and Takeda, 2004; Lebold et al., 2016; Ekman et al., 2011; Heil et al., 2004; Zhang et al., 2016).

TLR7/8 agonists can also be used to stimulate a strong immune response when used as vaccine adjuvants able to induce cell-mediated immunity (Tiboni et al., 2021). Bharat Biotech’s vaccine; Covaxin® developed in collaboration with the Indian Council of Medical Research (ICMR) and the National Institute of Virology (NIV), is similar to Sinovac® and Sinopharm® COVID-19 vaccines in that it is based on well-established vaccine technology and after inactivation, it is adjuvated with an imidazoquinoline (IMDg) class molecule (TLR7 and TLR8 agonist) chemosorbed on alum (Algel) (Algel-IMDG) (Tiboni et al., 2021). Moreover, it is to be mentioned that TLR7 agonists have previously demonstrated substantial utility as an infectious disease vaccine adjuvant, particularly for influenza (Hung et al., 2016; Weldon et al., 2012). Then, selectively targeting TLRs 7/8 would be beneficial in coping with the major challenge of current anti-COVID19 viruses which is viral escape (vaccine efficacy; 50–96%). The underlying immunostimulatory mechanism of new adjuvants targeting TLRs is by stimulating the innate immunity via production of cytokines/chemokines and type I interferons, followed by immediate development of adaptive immune responses so that host protection against the virus is strengthened and lasts longer (Khanmohammadi and Rezaei, 2021; Tiboni et al., 2021). A conceptual diagram illustrating the immuno-stimulatory effects of TLR7/8 agonists against SARS-CoV-2-induced mild-to-moderate COVID-19 is depicted in Fig. 1.

The fate of TLR7/8 agonists use as therapeutic targets will depend heavily on whether their use is associated with sustained systemic harms and intolerable sickness-related side effects. Recent studies demonstrated that TLR7 stimulation is capable of inducing acute sickness responses, but more studies are necessary to investigate the mechanisms and determine whether negative responses occur after acute or chronic use and whether such negative responses are self-limited or sustained (Michaelis et al., 2019). Some clinical trials with TLR7 agonists observed manifestations associated with acute sickness responses, including fever and flu-like symptoms (Sauder et al., 2003). Several small-molecule TLR7/8 agonists have also been clinically approved or are under ongoing investigation such as imiquimod (Rodell et al., 2019) with some side effects (headache, fever) that could hamper their clinical efficacy upon repeated systemic dosing (Pockros et al., 2007b). AZD8848 is also a novel TLR7 agonist that has been designed as an ante-drug to restrict the effects of the drug to the site of administration and to minimize any potential side effects associated with systemic cytokine production while being used for the treatment of asthma and allergic rhinitis (Leaker et al., 2019). It is a metabolically labile ester that is topically active but is rapidly hydrolysed by butyrylcholinesterase to a much less active metabolite upon entry into the circulation (Khan et al., 2005). One study investigated the efficacy, safety, and tolerability of intranasal AZD8848 60 μg administered once-weekly for 8 weeks in patients with physician-diagnosed mild allergic asthma who were subsequently challenged with an inhaled allergen. The drug was administered intranasally to minimize any risk of local inflammatory effects in the lungs and to further reduce the risk of the systemic activity. The results of this study revealed that the incidence of adverse events was similar in the two groups and that AZD8848 was generally well tolerated (Leaker et al., 2019). Murine models were also used to investigate acute and chronic effects of a therapeutically relevant dose of the imidazoquinoline TLR7/8 agonist R848 that is similar to the FDA-approved imiquimod but has a considerably higher potency. The results of this study revealed that the drug sickness responses are not protracted and do not result in cell death since they started following the initial dosing of TLR7/8 agonist and then receptor tolerance occurred thereafter and the drug did not continue to provoke behavioral or molecular evidence of inflammation. The lack of detected adverse outcomes in these studies further supports the notion that TLR7 agonists could be safely used in both acute and chronic applications (Michaelis et al., 2019).

1.2. TLR7/8 antagonists

From another perspective, TLRs were suggested to play key roles in the pathogenesis of the cytokine release syndrome and they were hypothesized to be the main PRRs participating in the induction of cytokine storm in the COVID-19-infected patients (Safaei and Karimi-Googheri, 2020; Karimi-Googheri and Arababadi, 2014). Stimulation of viral-sensing toll-like receptors in vitro and administration of synthetic
viral RNA in vivo induced features of hyper-inflammation, including cytokine elevation, immune cell airway infiltration, and MC-protease production (Gebremeskel et al., 2021). A Phase IIa trial on the psoriasis patients revealed that using IMO-3100, an agonist of TLR7, significantly modulated immune responses and down-regulated cytokine production in these patients (Kimball et al., 2013). Also, Merck as a leading science and technology company, announced that the U.S. Food and Drug Administration (FDA) has cleared its investigational new drug application (IND) for M5049, that blocks the activation of TLR7 and TLR8, for the treatment of patients with Covid-19 pneumonia (Merck, 2021). The company initiated a Phase II randomized, controlled clinical study evaluating the safety and efficacy of M5049 in this patient population at sites in the United States and Brazil based on the scientific hypothesis that activation of TLR7/8 leads to immune cell activation and inflammation, which when not properly controlled can cause severe immunopathology and that the intervention with the investigational drug may reduce life-threatening complications of Covid-19, including severe respiratory symptoms. Another study demonstrated that severe cases of COVID-19 differed from healthy controls and mild COVID-19 patients in exhibiting increased TLR7 activity (Root-Bernstein, 2021). Therefore, TLR7 and/or TLR8 antagonists could also be of value as add on therapies in severe cases of the disease since their corresponding TLR7 agonists were reported to induce high levels of Type-I interferons and the highest IFN-gamma levels were reported to be displayed by TLR8 and TLR7/8 agonists, which also induced the highest levels of pro-inflammatory cytokines IL-12, TNF-alpha, and IL-1 beta (Ghosh et al., 2006). In addition, TLR7 activation leads to the production of IL-1, IL-6, monocyte chemoattractant protein-1, MIP-1A, TNF-α, and type 1 IFN (Khanmohammadi and Rezaei, 2021; Yazdapanah et al., 2020) and in cultured neurons, it was also reported to induce IL-6 and TNF-α expression through Myd88 (Liu et al., 2013). The combined administration of TLR7 and/or TLR8 antagonists with other biologics and/or pharmacotherapies classicaly used to target the cytokine storm, may have a synergistic effect that could help in reducing the dose of such therapies and consequently their adverse effects. It may also help in reducing the length of stay in the ICU for severe COVID patients which will have positive pharmacoeconomic value. A conceptual diagram illustrating the immuno-suppressant effects of TLR7/8 antagonists against SARS-CoV-2-induced severe COVID-19 is depicted in Fig. 2.

Finally, TLR7/8 agonist/antagonist may play key roles in developing better drugs that may also be added to preventive vaccines, and combating early, as well as, late COVID-19 pathogenesis (Khanmohammadi and Rezaei, 2021; Chugh et al., 2021). The stage of the disease is important in targeting the condition with either the agonist or the antagonist of TLRs. Agonists of TLRs such as the innate immune stimulator, imiquimod, could be an option to control COVID-19 since it binds to TLR7/8 and induces the production of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-2, IL-6, IL-8, IL-12, as well as IFN-α. However, it should be mentioned that activation of TLR7/8 can induce a strong pro-inflammatory response in COVID patients, leading to acute lung injury and it may have a dual role in the progression of the disease (Moreno-Eutimio et al., 2020; Veras et al., 2020). It should also be noticed that imiquimod in the late stage of the infection could lead to cytokine storm and side effects of consistent inflammation. Therefore,
imiquimod is appropriate in the early stages of COVID-19 infection (Angelopoulou et al., 2020). The early stages of the disease or mild COVID should meet the case definition for COVID-19 without evidence of viral pneumonia or hypoxia and the late stage of the disease or severe COVID should have clinical signs of severe pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate >30 breaths/min; severe respiratory distress; or SpO2 < 90% on room air (World Health Organization, 2020).

Whole-genome sequencing of SARS-CoV, MERS-CoV, and SARS-CoV-2 revealed that TLR7 could be more involved in the pathogenesis of SARS-CoV-2 in comparison to SARS-CoV and MERS-CoV as it has more ssRNA motifs that could bind to TLR7 (van der Made et al., 2020). Nevertheless, clinical studies should determine the proper dose of TLR7 agonists to be used in the early stage of the disease if used as monotherapy or if combined with antiviral medications, since excessive activation of TLR7 is considered to be involved in the pathogenesis of several autoimmune diseases (Sourris et al., 2018). TLR7-specific antagonists such as Cpd-6 and Cpd-7 (Tojo et al., 2020), the TLR7/9 dual antagonists such as DV-1179 and IMO-3100, or the TLR7/8/9 antagonists such as IMO-8400 that are recently developed and were demonstrated to be very effective in reducing the dose of drug combinations may have potential synergistic effects and minimized toxicities; allowing for better pharmacological outcomes using reduced drug doses.

2. Conclusion

Pharmacological compounds acting on TLR7 and/or TLR8 could be of therapeutic value in COVID-19. TLR7 and/or TLR8 agonists may be used by frontline healthcare workers with comorbidities who demonstrate mild disease that could progress to severe manifestations, and as beneficial vaccine adjuvants as well. On the other hand, TLR7 and/or TLR8 antagonists may be used in combination with other immunomodulatory/anti-inflammatory drugs in severe cases of the disease. These drug combinations may have potential synergistic effects and minimized toxicities; allowing for better pharmacological outcomes using reduced drug doses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence...
the work reported in this paper.

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