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BRIEF COMMUNICATION

Chloroquine/hydroxychloroquine: an inflammasome inhibitor in severe COVID-19?

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Abstract

Chloroquine and hydroxychloroquine belong to the aminoquinoline drugs. Studies revealed that chloroquine and hydroxychloroquine shows antagonism activity against COVID-19 under laboratory conditions. ARDS and ALI are conditions that occur in patients with COVID-19 as the main pathological complications of cytokine storm. Inflammasomes play a key role in the pathogenesis of many diseases associated with destructive inflammation. NLRP3 inflammasome has been shown to play a key role in the pathogenesis of viral diseases. The possible role of NLRP3 inflammasome inhibitors in the treatment of COVID-19 has been considered. We surveyed the potential inhibitory effect of chloroquine and hydroxychloroquine on inflammasome. Studies indicate that one of the possible anti-inflammatory mechanisms of chloroquine and hydroxychloroquine is inhibition of the activity of NLRP3 inflammasome.

Keywords Chloroquine · Hydroxychloroquine · COVID-19 inflammasomes

Introduction

Chloroquine (CQ) and hydroxychloroquine (HCQ) belong to the aminoquinoline drugs. They originally have been produced as drugs against malaria, but are also capable to use for rheumatoid arthritis and other rheumatic diseases such as lupus. They are categorized as disease-modifying antirheumatic drugs (DMARDs). Unlike nonsteroidal anti-inflammatory drugs and steroids, these drugs not only eliminate the symptoms of the disease but also affect the course of the disease (Hickley et al. 2011; Taherian et al. 2013). Studies revealed that chloroquine shows antagonism activity against COVID-19 under laboratory conditions. However, evidence of its effects on patients is limited. The optimal role of these drugs, if any, has not yet been elucidated (Mehta et al. 2020).

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The SARS-CoV-2 virus, which belongs to the beta-coronavirus, can cause severe respiratory syndrome by involving the lower respiratory tract. Clinically, it is associated with symptoms such as fever, cough, muscle aches, fatigue, diarrhea, and pneumonia, and in severe cases can lead to death. One of the leading causes of death in patients with COVID-19 is a phenomenon called a cytokine storm. A group of patients shows severe symptoms of the disease. Acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) are conditions that occur in patients with COVID-19 as the main pathological complications of cytokine storm (Phua et al. 2020). Inflammasomes are one of the most important innate immune components that enhance inflammation by increasing the production of IL-1β, IL-18, and gasdermin. Inflammasomes play a key role in the pathogenesis of many diseases associated with destructive inflammation. In viral infections, numerous studies have shown that inflammasome is overactive, resulting in destructive and systemic inflammation in patients. NLRP3 inflammasome has been shown to play a key role in the pathogenesis of viral diseases (Zhao and Zhao 2020; de Castro-Jorge et al. 2019; Shrivastava et al. 2016). The proliferation of SARS-CoV-2 in a wide range of cells can be combined with numerous observations of direct and indirect activation of inflammasomes by other coronaviruses. Inflammation activation by inflammasomes is likely to be involved in the development of severe cytokine storms, which

subsequently cause ARDS and dysfunction of various organs and ultimately lead to the patient's death. SARS-CoV-2 encodes ion channel proteins called viroporins, such as the E, ORF3a, and ORF8a proteins. These viroporins activate the NLRP3 signaling receptor through mechanisms such as lysosomal malfunction and ionic redistribution in the intracellular environment. The possible role of NLRP3 inflammasome inhibitors in the treatment of COVID-19 has been considered. Due to the clinical use of several NLRP3 inhibitors for the treatment of other inflammatory diseases, controlled studies on COVID-19 patients have been suggested or considered to be effective in the treatment of COVID-19 (Shah 2020).

The effect of CQ and HCQ on NLRP3 inflammasome activation

In a study, the researcher investigated how chloroquine suppresses the activation of NLRP3 inflammasome and protects the mice against endotoxic shock. Chloroquine in mouse bone marrow-derived macrophages (BMDMs) reduced the activity of NF-κB and MAPK and inhibited IL-1β, IL-18, and NLRP3 expression, indicating its inhibitory effect on the NLRP3 activator initiation signal. Chloroquine inhibited the activation of caspase-1 and the formation of ASC complexes in BMDMs, indicating that chloroquine also inhibits the formation of the inflammasome complex (the second signal to activate NLRP3 inflammasome) (Chen et al. 2017). In the mice model of endotoxic shock, chloroquine effectively improved survival, and significantly reduced IL-1β and IL-18 production in serum, peritoneal fluid, and lung tissue. Also, chloroquine reduced the levels of the NLRP3 protein and caspases-1 p10 in homogenates in the lungs of mice with endotoxic shock, which could explain its anti-inflammatory activity and protective effects within the body (Chen et al. 2017) .In one study, mice were exposed to ischemia-reperfusion (I/R) damage and received hydroxychloroquine with gavage route for 7 days before the I/R surgery. In parallel, HK-2 human renal proximal tubule cells (RPTC) were received hydroxychloroquine as prophylaxis and then exposed to hypoxia/re-oxygenation (H/R). The results showed that hydroxychloroquine significantly reduces renal dysfunction by reducing serum creatinine, reducing the expression of protein molecular-1 kidney damage (KIM-1), and improving HK-2 cell viability. Also, hydroxychloroquine significantly reduces macrophage and neutrophil infiltration, the production of inflammatory cytokines, and the activation of NLRP3 inflammasome. Hydroxychloroquine can reduce the initial signal of the inflammasome by reducing the expression of NF-κB signaling induced by I/R or H/R (Tang et al. 2018). Artemisinin and hydroxychloroquine both have anti-inflammatory and immune-modulatory activities. The pharmacological effects of artemisinin in combination with hydroxychloroquine and their potential molecular mechanisms in IgA nephropathy were studied. In vivo, artemisinin and hydroxychloroquine can effectively improve kidney damage by improving renal dysfunction and reducing the levels of 24-h urinary protein, IgA and IgG, and eliminating IgG immune complexes in IgA nephropathy mouse glomeruli. Artemisinin and hydroxychloroquine specifically accelerate the secretion of exosomes in renal tissues, inhibiting NF-κB signaling and production of NLRP3 inflammasome-related proteins, including IkB-α, p-p65, NLRP3, ASC, caspase-1, and IL-1β in mice with IgA nephropathy (Bai et al. 2019). In vitro evaluation, exosomes of human kidney epithelial cells increased significantly by artemisinin and hydroxychloroquine, which can be fully collected in human mesangial cells (HMCs) and inhibit activation of the NF-kB path and NLRP3 inflammasome. An inhibitory effect of artemisinin and hydroxychloroquine treatment in NF-κB signaling and NLRP3 by releasing exosomes in mice with IgA nephropathy is an alternative method for the treatment of IgA nephropathy (Bai et al. 2019). Although hydroxychloroquine is a known ion channel inhibitor, this is not related to its anti-inflammatory effects. A study was conducted to investigate whether hydroxychloroquine inhibits anti-inflammatory ion channels or not. In macrophages, ATP-induced K + efflux plays an important role in activating the NLRP3 inflammasome. In vitro evaluation, hydroxychloroquine inhibits the activation of IL-1β and caspase-1 induced via ATP by the dose-dependent method. Hydroxychloroquine reduces ATP-induced K + efflux. Hydroxychloroquine also inhibits the use of ATP-induced and caspase-1-related neutrophils. Hydroxychloroquine inhibits activated K + and Ca ++ channels. This effect is likely to interfere with the activation of inflammasome (Schroeder et al. 2017). To study the effect of hydroxychloroquine on NLRP3 inflammasome, human neutrophils were stimulated by serum amyloid A (SAA), and IL-1β and caspase-1 (p20) secretion measured. The amount of mRNA expression of the Pro-IL-1b gene was also measured in human neutrophils. Pre-treatment with hydroxychloroquine significantly inhibited the production of SAA-induced IL-1β in human neutrophils, but the activation of SAA-induced NF-κB, expression of pro-IL-1β mRNA, and NLRP3 protein did not get affected. Also, SAA stimulation induced the cleaved of caspase-1 (p20) by human neutrophils, which is suppressed by pre-treatment of hydroxychloroquine. Treatment with hydroxychloroquine was associated with impaired IL-1β production in SAA-stimulated human neutrophils without affecting the initial activation process of NLRP3 inflammasome, such as pro-IL-1β induction or NLRP3 induction. These findings suggest that hydroxychloroquine affects the activation process of NLRP3, and reduces the production of IL-1β in human neutrophils as the innate immune cells (Fujita et al. 2019).

Other drugs to inhibit the NLRP3 inflammasome

In this study, although we focused on CQ and HCQ as of the most important and, of course, the most challenging drugs in the treatment of COVID-19, a range of drugs including statins, antiviral drugs, interferons, antiallergic compounds, and immunomodulators are currently being used in clinical trials on COVID-19. In addition to their better known and primary roles, they inhibit the activation of NLRP3 inflammasome. Because of the challenges and controversy surrounding the effectiveness of HCQ and CQ, it is important to consider other drugs used to inhibit NLRP3 inflammasome, some of which will be reviewed below. Statins have been proposed as therapeutic agents in different diseases including infections such as influenza virus or MERS-CoV (Phadke and Saunik 2020). The well-known anti-inflammatory properties of statins, by blocking several molecular mechanisms, including NF-κB and NLRP3 inflammasome, could limit the cytokine storm in severe COVID-19 patients (Rodrigues-Diez et al. 2020). The interferons are of the key mechanisms in antiviral immunity. Numerous studies demonstrated that beta-coronaviruses such as SARS-CoV, MERS-CoV, and SARS-CoV-2 inhibit the interferon-based immune responses in a variety of ways (Lieberman et al. 2020). Therefore, like HCQ and CQ, several clinical trials are underway to evaluate the effectiveness of recombinant interferons in the treatment of patients with COVID-19. However, studies have shown that the interferons, especially type-I IFN, inhibit the NLRP3 inflammasome in various mechanisms (Mishra et al. 2013; Kopitar-Jerala 2017). Some trials are studying the efficacy of lopinavir/ritonavir and IFNs (ChiCTR2000029387) or the combination of lopinavir/ritonavir/ribavirin (NCT04276688, EudraCT 2020-001023-14). The inhibitory effects on the NLRP3 inflammasome, in addition to the immunomodulators of HCQ and CQ, have been reported for some antiviral drugs such as protease inhibitors (ritonavir and lopinavir). Numerous clinical trials (such as NCT04255017, NCT04307693NCT04315948, and NCT04328285) are being conducted in different countries to evaluate the effectiveness of ritonavir and lopinavir in the treatment of COVID-19. Darunavir is another protease inhibitor that is being evaluated as an antiviral drug in the treatment of COVID-19 (NCT04252274, ChiCTR2000029541, NCT04261270, and NCT04303299). Despite focusing on the protease inhibitor properties of these three antiviral compounds, studies have shown that all three of these compounds are able to inhibit the activation of the NLRP3 inflammasome (Weichold et al. 1999). Clinical trials are being performed to evaluate the effect of azithromycin alone or in combination with HCQ in the treatment of COVID-19 (NCT04332107, NCT04381962, NCT04369365, NCT04371107NCT04339816, and NCT04336332). Like HCQ, studies have shown the role of azithromycin in inhibiting severe neutrophilic inflammation by inhibiting the activation of NLRP3 inflammasome (Gualdoni et al. 2015; Lendermon et al. 2017). Additionally, the immunosuppressants are also considered as potential treatments in severe cases of COVID-19. Sirolimus is one of the most well-known immunosuppressants whose effectiveness in the treatment of COVID-19 has been studying in several clinical trials (NCT04341675NCT04371640 and NCT04374903). Another inhibitor is cyclosporine, which is currently being examined in the clinical trials to treat the COVID-19 infection through the control of cytokine storms and to improve disease prognosis alone and in combination with other drugs (NCT04412785, NCT04392531, NCT04540926, and NCT04341038). Both of these immunosuppressants, along with their main mechanism, are able to interfere with the activation of the NLRP3 inflammasome (Iyer et al. 2013; Ko et al. 2017). Colchicine is another drug widely used in clinical trials on COVID-19 (NCT04392141, 2020-001511-25, NCT04322565 NCT04375202, and NCT04360980), which is able to inhibit NLRP3 inflammasome in various ways, such as preventing the caspase activation, inhibiting the P2X7 receptor, decreasing the pyrine gene expression and thus inhibiting the formation of the NLRP3 inflammasome complex (Otani et al. 2016; Marques-da-Silva et al. 2011). In addition to HCQ, JAK inhibitors and Bruton tyrosine kinase (BTK) inhibitors such as baricitinib and acalabrutinib are other compounds tested in the treatment of COVID-19. Some clinical trials related to baricitinib (NCT04373044, NCT04393051, NCT04320277, and NCT04393051) and acalabrutinib (NCT04497948, NCT04380688, and NCT04564040) have been registered on ClinicalTrials.gov. Studies have shown that both of these compounds inhibit the necessary components for the formation of the NLRP3 inflammasome complex by interfering with signaling pathways (Furuya et al. 2018; Collotta et al. 2020; Purvis et al. 2020). Another drug used in the clinical trials on COVID-19 is dexamethasone, dozens of which are currently registered only on ClinicalTrials.gov (NCT04499313, NCT04395105, and NCT04344730). This drug, in addition to its anti-inflammatory effects, also reduces pulmonary inflammation by inhibiting NLRP3 inflammasome in the lungs. The main inhibitors of inflammasome products such as canakinumab and anakinra are being studied in more than a dozen clinical trials to help treat COVID-19. On the other hand, a significant increase in IL-6 level has been observed as a marker for the cytokine storm in the COVID-19 infection under conditions resulting from the hyperactivity of NLRP3 inflammasome (Abbate et al. 2020). IL1 beta increases IL-6 secretion, and in turn, inhibition of IL-6 signaling inhibits inflammasome activation. Thus, IL-6 inhibitors are able to effectively inhibit lethal inflammation and cytokine storms by inhibiting NLRP3 inflammasome. Sarilumab and tocilizumab are being evaluated as two anti-IL-6 receptor monoclonal antibodies in several trials in the treatment of COVID-19.

Conclusion

Hundreds of clinical trials are being conducted in different countries to investigate the potential treatments for COVID-19.The priority of many of these trials is to evaluate the effectiveness of drugs that have previously been used to treat other diseases in humans. Hydroxychloroquine has been used in recent years in the treatment of many autoimmune disorders as a drug with immunomodulatory properties. It is also important to understand the mechanism of action of hydroxychloroquine in this regard. Chloroquine and hydroxychloroquine drugs are associated with side effects such as nausea, diarrhea, vomiting, retinopathy, and drug interactions that can lead to long QT and heart problems. We surveyed the potential inhibitory effect of hydroxychloroquine on inflammasome. Studies indicate that one of the possible anti-inflammatory mechanisms of hydroxychloroquine is inhibition of the activity of NLRP3 inflammasome. Understanding the exact mechanism of action of this drug can lead to its proper use and increase its effectiveness.

Authors' contributions ASB, MB, and MMS designed research. MMS and BK conducted experiments. ASB and MB analyzed data. MMS and BK wrote the manuscript. All authors read and approved the manuscript. All experimental data were generated in-house and we did not use a paper mill.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors.

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