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COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression

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A novel coronavirus disease (COVID-19), caused by infection with SARS-CoV-2, has swept across 31 provinces in China and over 40 countries worldwide. The transition from first symptoms to acute respiratory distress syndrome (ARDS) is highly likely to be due to uncontrolled cytokine release. There is an urgent need to identify safe and effective drugs for treatment. Chloroquine (CQ) exhibits a promising inhibitory effect. However, the clinical use of CQ can cause severe side effects. We propose that hydroxychloroquine (HCQ), which exhibits an antiviral effect highly similar to that of CQ, could serve as a better therapeutic approach. HCQ is likely to attenuate the severe progression of COVID-19, inhibiting the cytokine storm by suppressing T cell activation. It has a safer clinical profile and is suitable for those who are pregnant. It is cheaper and more readily available in China. We herein strongly urge that clinical trials are performed to assess the preventive effects of HCQ in both disease infection and progression.

SARS-CoV-2 and the COVID-19 outbreak

A new pathogen, identified as a novel coronavirus (SARS-CoV-2), triggered a novel pneumonia (COVID-19) outbreak in December 2019, starting in Wuhan, China and spreading quickly to 31 provinces in China and more than 40 countries worldwide. SARS-CoV-2 is a betacoronavirus and shares genetic sequence and viral structure with both severe acute respiratory syndrome coronavirus (SARS-CoV; 70% similarity), which caused 349 deaths during 2002–03 in China, and Middle East respiratory syndrome coronavirus (MERS-CoV; 40% similarity).[1] As of 3 March 2020, a total of 80 302 confirmed cases of COVID-19, including 2947 deaths, had been reported in mainland China, Hong Kong, Macao and Taiwan. A rapidly growing number of COVID-19 cases had also been reported from Japan, South Korea and Italy.

The number of COVID-19 cases is still on the rise. The median time from first symptoms to ARDS is 8 days (IQR 6–12 days).[2] The transition to ARDS occurs in many severe COVID-19 cases. A possible explanation for this rapid and serious deterioration is the cytokine release syndrome (CRS), or 'cytokine storm', an overproduction of immune cells and cytokines that leads to rapid multi-organ system failure and fetal damage to tissues of the lungs, kidney and heart.[3] Developing an effective approach to modulate the immune response or suppress overreactive cytokine production is of crucial importance in reducing disease aggravation and the mortality rate. There is an urgent need to identify effective and safe medical agents to treat this disease.

Antivirals

Several antiviral drugs have been tested for efficacy in inhibiting SARS-CoV-2 replication in cell culture. Two drugs have exhibited a promising inhibitory effect: remdesivir (GS-5734), an experimental drug being developed for the treatment of Ebola virus infection; and chloroquine (CQ), a drug well-known for its effectiveness in treating malarial and autoimmune disease.[4] Notably, remdesivir has demonstrated antiviral activity in treating MERS and SARS in animal models, both of which are caused by coronaviruses.[5] As an emergent therapeutic approach, approval was given for remdesivir to have its treatment effect tested in a group of COVID-19 patients. The clinical trial is currently ongoing at several hospitals in Wuhan and the results regarding effectiveness and safety are awaited. The EC_{90} of CQ for SARS-CoV-2 in Vero E6 cells is 6.90 μM,[4] which is clinically achievable, well tolerated in patients with rheumatoid arthritis and potentially applicable to COVID-19 patients. Several randomized controlled trials have been conducted to test the effect of CQ in treating COVID-19. Therapeutic effects were observed in aspects of fever reduction, improvements on CT imaging and retarded disease progression. CQ has been officially declared as a medical agent for COVID-19 in the sixth edition of the *New coronavirus pneumonia diagnosis and treatment plan*, released by the National Health and Care Commission of China on 19 February 2020. The recommended dosage for adults is 500 mg/day, which is the maximum sustainable dosage in the human body.

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Hydroxychloroquine (HCQ)

With a chemical structure very similar to that of CQ (Figure 1), HCQ is one of the disease-modifying antirheumatic drugs (DMARDs). The DMARDs are widely used for treating many rheumatic diseases and also demonstrate a strong immunomodulatory capacity, which prevents inflammation flare-ups and organ damage.[6] HCQ and CQ are considered to be immunomodulators rather than immunosuppressants (Figure 2). In particular, HCQ can increase the intracellular pH and inhibit lysosomal activity in antigen-presenting cells (APCs), including plasmacytoid dendritic cells (pDCs) and B cells, so preventing antigen processing and major histocompatibility complex (MHC) class II-mediated autoantigen presentation to T cells.[7] This process reduces T cell activation, differentiation and expression of co-stimulatory proteins (e.g. CD154 on CD4+ T cells)[8] and cytokines produced by T cells and B cells (e.g. IL-1, IL-6 and TNF).[9] Meanwhile, due to the altered pH of endosomes and interrupted binding between toll-like receptors (TLR7 and TLR9) and their RNA/DNA ligands, TLR signalling is suppressed by administration of HCQ.[10–13] In the cytoplasm, HCQ also interferes with the interaction between cytosolic DNA and the nucleic acid sensor cyclic GMP-AMP (cGAMP) synthase (cGAS).[14] As both TLR signalling and cGAS stimulation of interferon genes (the STING pathway) are impeded by HCQ, subsequent pro-inflammatory signalling activation and production of cytokines, such as type I interferons, IL-1 and TNF, are attenuated.[9] Such mechanisms give strong support to the hypothesis that HCQ is likely to confer an ability to suppress the CRS, which is due to overactivation of the immune system triggered by SARS-CoV-2 infection, through which progression of the disease from mild to severe might be attenuated. Therefore, careful clinical examination is urgently required to validate this hypothesis.

In addition to a role in immune modulation, HCQ and CQ inhibit receptor binding and membrane fusion, two key steps that are required for cell entry by coronaviruses. CQ has been shown to exert an antiviral effect during pre- and post-infection conditions by interfering with the glycosylation of angiotensin-converting enzyme 2 (ACE2) (the cellular receptor of SARS-CoV) and blocking virus fusion with the host cell (Figure 2). Impaired terminal glycosylation of ACE2 may reduce the binding efficiency between ACE2 on host cells and the SARS-CoV spike protein. Thus, the binding of the virus to the receptors on the cells is impeded and infection is consequently prevented. Once HCQ and CQ enter a cell they are both concentrated in organelles with low pH, such as endosomes, Golgi vesicles and lysosomes. As the virus uses endosomes as a cellular entry mechanism, increasing the pH of endosomes through CQ treatment places a negative influence on the fusion process of virus and endosome.[15] Lysosomal proteases activate the fusion process between host and viral membranes by cleaving coronavirus surface spike proteins.[16] Increasing the pH of the lysosome prevents protease activity such that this fusion process is disrupted.[17] Without the pH necessary for the endosome and lysosome to execute the cleavage function, replication of and infection by the coronavirus are blocked.[18] Inhibition of SARS-CoV spread was observed in cells treated with CQ prior to or after infection, suggesting both prophylactic and therapeutic advantages of CQ in combating SARS-CoV.[15] Given that HCQ demonstrates similar molecular mechanisms to CQ, it is highly likely that HCQ will perform similarly in terms of early prevention and disease progression. Again, this requires careful *in vitro* and *in vivo* testing.

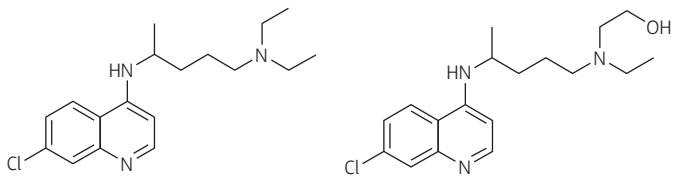

Figure 1. The chemical structures of CQ and HCQ.

Safety and adverse events

Both HCQ and CQ have a good safety record and are well distributed throughout the whole body after oral administration, especially in acidic compartments such as lysosomes and inflamed tissues. Another advantage of HCQ and CQ is that they do not bring risks of infectious complications, unlike immunosuppressant drugs such as methotrexate and leflunomide.[19] Gastrointestinal responses, such as vomiting and diarrhoea, are the most common adverse effects of these two drugs.[20] Patients with long-term exposure to CQ suffer from severe side effects, such as retinopathy, circular defects (or bull's eye maculopathy), diametric defects in the retina and cardiomyopathy.[17] Elderly patients and usage beyond dosage limits are also associated with toxicities of CQ therapy. In contrast, HCQ has a lower level of tissue accumulation,[17] which may explain the fact that it is associated with fewer adverse events than CQ, but still potentially influences the prevention and treatment of malaria to a similar level. Indeed, only high-dose and long-term (over 5 years) intake of HCQ is likely to contribute to the development of retinopathy,[21] which is in agreement with the current preference of HCQ use in therapy. While CQ exerts a number of severe side effects on fetal development, HCQ is strongly recommended for pregnant patients with an autoimmune disease as it prevents the development of congenital heart block due to a potential inhibitory effect of type I interferon production.[22,23] The outbreak of SARS-CoV-2 has placed many pregnant women at high risk of infection (several infected cases have been reported); HCQ, rather than CQ, should be considered as a potential therapeutic solution for these patients, given its safety profile in pregnancy. More importantly, the maximum tolerable dose for HCQ is 1200 mg, which has an antiviral effect equivalent to 750 mg CQ (for which the maximum tolerable dose is 500 mg).[24] This indicates that HCQ can be administered at a higher dosage and may therefore achieve a more powerful antiviral effect.

Conclusions

In summary, we propose that HCQ could serve as a better therapeutic approach than CQ for the treatment of SARS-CoV-2 infection. There are three major reasons for this: (i) HCQ is likely to attenuate the severe progression of COVID-19 through inhibiting the cytokine storm by reducing CD154 expression in T cells; (ii) HCQ may confer a similar antiviral effect at both pre- and post- infection stages, as found with CQ; (iii) HCQ has fewer side effects, is safe in pregnancy and is cheaper and more highly available in China. Given the fast-growing number of COVID-19 patients and the

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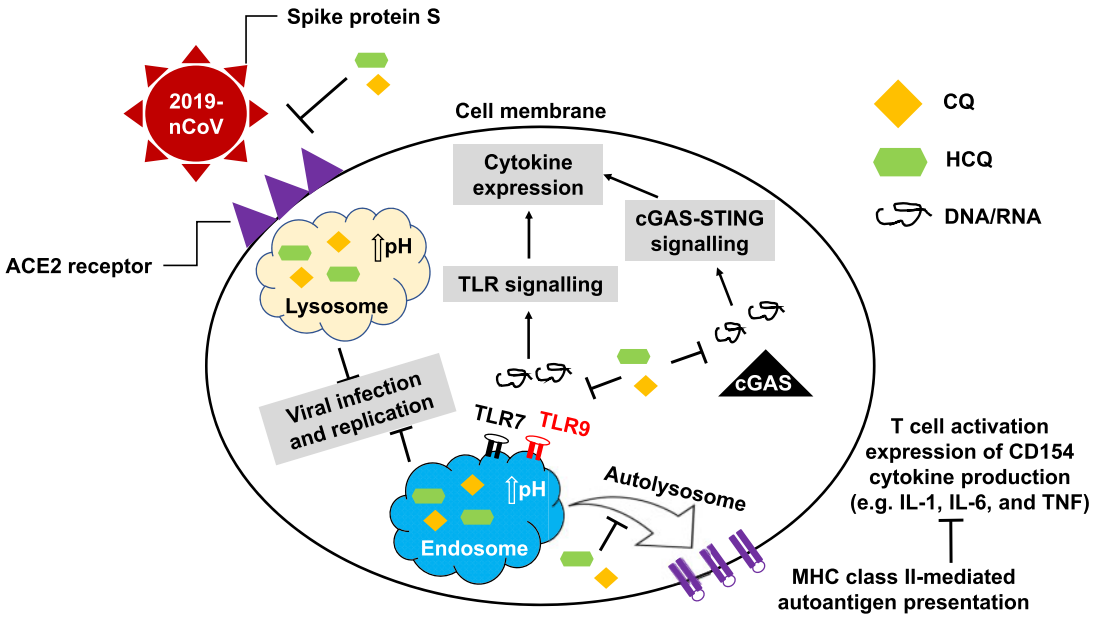

Figure 2. A graphical illustration of the antiviral mechanisms of CQ and HCQ. Both chemicals can interfere with the glycosylation of ACE2 and reduce the binding efficiency between ACE2 on the host cells and the spike protein on the surface of the coronavirus. They can also increase the pH of endosomes and lysosomes, through which the fusion process of the virus with host cells and subsequent replication are prevented. When HCQ enters APCs, it prevents antigen processing and MHC class II-mediated autoantigen presentation to T cells. The subsequent activation of T cells and expression of CD154 and other cytokines are repressed. In addition, HCQ disrupts the interaction of DNA/RNA with TLRs and the nucleic acid sensor cGAS and therefore the transcription of pro-inflammatory genes cannot be stimulated. As a result, administration of CQ or HCQ not only blocks the invasion and replication of coronavirus, but also attenuates the possibility of cytokine storm. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

urgent need for effective and safe drugs in the clinic, it is more practical to identify reliable candidates by screening currently available drugs. We herein strongly urge that clinical trials are performed to assess the preventive effects of HCQ on both infection and malignant progression.

Transparency declarations

None to declare.

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