Efficacies of repurposing chloroquine analogues for the treatment of COVID-19: Facts and myths

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Abstract

The emergence of coronavirus disease 2019 (COVID-19) is caused by the 2019 novel coronavirus (2019-nCoV). The 2019-nCoV first broke out in Wuhan and subsequently spread worldwide owing to its extreme transmission efficiency. The fact that the COVID-19 cases and mortalities are reported in globally and the WHO has declared this outbreak as the pandemic. The international health authorities have focused on rapid diagnosis and isolation of patients as well as the search for therapies able to counter the disease severity. Due to the lack of a known efficient therapy and public health emergency, repurposing drugs chloroquine (CQ) analogues appear to be the best tool against 2019-nCoV infection. These analogues have shown potential efficacy to inhibit 2019-nCoV in vitro that leads to focus in several new trials. This review discusses the possible effective roles and mechanisms of CQ analogues for interfering with the 2019-nCoV replication cycle and infection.

Introduction

Coronaviruses (CoVs) are members of the family *Coronaviridae* in the order *Nidovirales* .[1] CoVs are enveloped, single-stranded, positive-sense RNA viruses having the largest genome among RNA viruses (˜32kb).[2] CoVs are spreading worldwide and naturally infect a variety of species. Earlier, six CoVs have been identified that mostly cause respiratory and central nervous system (CNS) pathologies in humans. Human coronavirus 229E (HCoV-229E) and HCoV-OC43 classified in the genus of*Alpha-coronavirus* and *Beta-coronavirus* lineage 2a member respectively, usually cause common colds during the winter and early spring.[3,4] HCoV-NL63 (*Alpha-coronavirus* lineage 1b member) is responsible for causing croup in children and outcomes in more severe clinical features than those of HCoV-229E or HCoV-OC43.[4-6] HCoV-HKU1 (*Beta-coronavirus*lineage 2a member) is associated with bronchiolitis and pneumonia.[7] In 2003, a lethal zoonotic CoV infection called severe acute respiratory syndrome (SARS) was reported to outbreak in China and associated with SARS-CoV-1 (*Beta-coronavirus* lineage 2b member).[8] In 2012, a SARS-like disease re-emerged and the causative agent was identified as Middle East respiratory syndrome CoV (MERS-CoV, as classified in *Beta-coronavirus* lineage 2c).[9,10] All of the human CoVs are infectious respiratory pathogens that also cause neurological diseases.[11-13] Thus, all of the CoVs are thought to be responsible for respiratory tract infections and neurological pathologies in a similar way to other known neuroinvasive viruses like measles virus or human immunodeficiency virus (HIV).[14]Recently, the seventh human CoV called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as officially named as 2019 novel coronavirus (2019-nCoV) a novel *Beta-coronavirus* emerged in the Chinese city of Wuhan in December 2019 as being responsible for respiratory infection including pneumonia.[15,16]

Depending on drastic outbreak measures and the worldwide spreading because of its probable high transmission efficiency, 2019-nCoV is also known as SARS coronavirus 2 (SARS-CoV-2). Phylogenetic analysis of this virus indicates that it is different (˜80% nucleotide identity) but related to SARS-CoV-1.[17] In clinical pathology, 2019-nCoV is responsible for respiratory infections including asymptomatic carrier state, acute respiratory disease (ARD) and pneumonia with a mortality rate estimated about 2%-2.5%, increasing with

age and the existence of underlying diseases.[18]Since the 2019-nCoV has transmitted with high frequency, and multiple countries have confirmed the coronavirus disease 2019 (COVID-19) cases,[19-21] the World Health Organization (WHO) has announced a new name as pandemic disease on March 12th2020.[22]

COVID-19 is now public health emergency of international concern. Currently, the world is threatened by the COVID-19 pandemic because the number of people diagnosed with 2019-nCoV infection is increasing day by day. Unfortunately, to date, no known validated specific, efficient therapies have approved by drug regulatory agencies for the treatment of 2019-nCoV infection. Scientists are endeavouring to discover drugs or vaccines to treat this disease, but it takes much more times. Thus, the researchers and medical teams have focused on repurposing FDA-approved drugs to treat the most vulnerable and severe cases of this infection.[23] Drug repurposing is an effective way to quickly identify therapeutic drug with a known safety profile to treat an emerging disease. As these aspects, chloroquine (CQ) analogues, traditionally used to treat malaria, are necessary particular attention for repurposing for COVID-19 treatment because of their broad-spectrum antiviral effects. CQ analogues have been shown to inhibit effectively the viral replication including SARS-CoV and MERS-CoV.[24-26] Recently, CQ is found to inhibit 2019-nCoV *in vitro* and its hydroxylated form, hydroxychloroquine (HCQ) has been proposed as a possible therapy to treat patients infected with 2019-nCoV.[26,27] Based on *in vitro* results and clinical studies in several Chinese hospitals, a great deal of effort has been made to find effective drugs against the virus in China.[28] On February 17, 2020, the State Council of China provided a briefing news indicating that the superiority, marked efficacy and acceptable safety of CQ analogue in treating COVID-19 in terms of reduction of exacerbation of pneumonia, duration of symptoms and delay of viral clearance.[29] These results have directed in China to include CQ analogue in the recommendations regarding the prevention and treatment of COVID-19 pneumonia.[29-31]

Repurposing chloroquine (CQ) analogues: A hurdling therapy from malaria to viral diseases

Nobel laureate (1988) James Black, the renowned pharmacologist precisely defines the repositioning approach as "the most fruitful basis for the discovery of a new drug is to start with an old drug". The traditional drug discovery is an indeed challenging field in terms of rising and unsustainable costs (several hundred million dollars expenses from an idea to a marketed drug), and time-consuming tasks (an average of 15 years), with a high failure rate.[32] Pharmaceutical industries have experienced to an annual decrease in return on investment[33] and health care authorities have faced the prime challenge in their existence for financial sustainability fuelled by the costs of prescription drugs.[34] In this context, drug repurposing (scanning the existing pharmacopoeia for new therapeutic uses or indications) appears as a new value proposition for the industry, patients and payers.[35,36] Moreover, the repurposing approach may overcome many problems associated with producing new drugs by the traditional method because of their known pharmacokinetics, pharmacodynamics, and safety profiles and approved by the regulatory agencies, for example the Food and Drug Administration, USA (FDA) and this knowledge accelerates the evaluation of the drug in clinical trials.[37,38]

Historically, one of the well-known repurposing victory stories is the (re)use of CQ analogues. CQ and its structural analogues such as HCQ, pamaquine, plasmoquine, primaquine or mefloquine have been used for decades as the primary and most successful drugs against malaria. These analogues still remain the drug of choice for malaria chemotherapy because they are inexpensive, orally available, well tolerated and effective drugs and low toxicity in humans.[39]Concomitant with the emergence of CQ-resistant *Plasmodium* strains and a subsequent decrease in the use as antimalarial drugs, new potential uses of the cheap and existing analogues have also been investigated.[39] Due to their immunomodulatory effects, these analogues have been used as secondary drugs to treat a variety of chronic autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus etc.), tumors and nonmalarial infections (Table 1).[39] Recently, several efforts have been made to identify effective, inexpensive and universally available antiviral agents (Table 1). In these aspects, the CQ analogues have been suggested as such antiviral agents by inhibiting several viral replications.[25,40] For examples, these analogues are explored against filoviral infections including influenza A and B, SARS coronavirus, hepatitis A virus and the Borna disease and other viral diseases such as HIV and dengue, MERS virus and chikungunya virus (CHIKV) which are associated with replication cycle and inflammation.[41] Thus,

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the repurposing approach of CQ analogues has been successfully passed by serendipitous discovery or clinical observation.

Antiviral activities of chloroquine (CQ) analogues

The antiviral activity of CQ analogues has been identified since the late 1960's.[41] CQ analogues suppress the growth of versatile viruses (Table 2) in cell cultures *in vitro* e.g., the SARS coronavirus.[42] These analogues have been reported to possess antiviral activity against a wide range of RNA viruses[43,44] such as HIV, hepatitis A virus, hepatitis C virus, influenza A H5N1 virus,[45] influenza A and B viruses, CHIKV,[46] Dengue virus,[47]Crimean–Congo hemorrhagic fever virus,[48] Ebola virus, Zika virus, Hendra and Nipah viruses.[41]

In case of acute infection like CHIKV, although CQ shows promising antiviral activity *in vitro* ,[46,49] but is shown to augment alphavirus replication in various animal models *in vivo* .[50,51] In a nonhuman primate model of CHIKV infection, CQ treatment is also shown to exacerbate acute fever and delay the cellular immune response, leading to an incomplete viral clearance.[50] A clinical trial conducted during the chikungunya outbreak in 2006 in Réunion Island demonstrates that oral CQ treatment is powerless to improve the course of this acute disease[51] and that chronic arthralgia on day 300 post illness is more frequent in treated patients than in the control group.[50] Altogether, the evaluation of previous data suggests that CQ analogues have lack of capacity to inhibit acute virus infection successfully in humans. CQ analogue has also been assessed in chronic viral diseases. Its use in the treatment of HIV-infected patients has been considered in conclusive[52] and the drug has not been included in the panel recommended for HIV treatment. The only modest effect of CQ in the therapy of human virus infection is found for chronic hepatitis C.[53,54] This is not enough to include CQ in the standardised therapeutic protocols for hepatitis C patients. Interestingly, a line of evidence indicates that the antiviral activity of these analogues is found in mice against a variety of viruses, including human coronavirus OC43,[55] Zika virus[56] and influenza A H5N1.[45]However, CQ is also active *ex vivo* but not *in vivo* in the case of Ebola virus in mice,[41] and influenza virus[57] in ferrets. Moreover, CQ analogue is incapable to prevent influenza infection in a randomized, double-blind, placebo-controlled clinical trial[41] and has no effect on Dengue-infected patients in a randomized controlled trial in Vietnam.[58] Thus, the antiviral properties of CQ analogues described *in vitro* have sometimes been established during treatment of virus-infected patients but have not always been reproduced in clinical trials depending on several factors such as the severity of disease, the concentration of CQ analogue used, the duration of treatment and the clinical team in charge of the trials.

Potentiality of chloroquine (CQ) analogues against coronaviruses including 2019-nCoV

CQ analogues have broad spectrum of antiviral action against coronaviruses (Table 2). For example, the potential therapeutic benefits of CQ analogues are notably reported for SARS-CoV-1.[25,55] CQ analogue is also reported to inhibit *in vitro* the replication of HCoV-229E in epithelial lung cell cultures.[59] Also, the administration of CQ through the mother's milk averts lethal infections of HCoV-O43 coronavirus in newborn mice.[41] Moreover, a strong antiviral effect of CQ is exhibited on a recombinant HCoV-O43 coronavirus i*n vitro* .[60] Although CQ analogue is reported to be active against MERS-CoV *in vitro* ,[61] this observation remains controversial.[62] 2019-nCoV (SARS-CoV-2) is closely relative to SARS-CoV-1 due to the occurrence of cell entry and replication cycle through the endolysosomal pathway.[43,63] Thus, it makes sense in a public-health emergency situation and the absence of any known efficient therapy, it is indispensable to investigate the possible antiviral effects of these analogue against 2019-nCoV. A recent paper indicates that CQ analogue is found to inhibit the growth of 2019-nCoV *in vitro,* [27] and suggests these drugs be assessed in human patients suffering from COVID-19.[64] According to preliminary reports,[29,31] the National Centre for Biotechnology Development (NCBD), China designated that CQ analogue is one of the most promising drugs against the new 2019-nCoV coronavirus that causes COVID-19. These findings have been supported that approximately one-hundred 2019-nCoV infected patients treated with CQ analogue experienced a more rapid decline in fever and improvement of lung computed tomography (CT) images and required a shorter time to recover compared with control groups, with no obvious serious adverse effects. Thus, the Chinese medical advisory board and authorities have suggested CQ analogue inclusion in the 2019-nCoV treatment guidelines. As a result, CQ is probably the first molecule to be used in China and abroad on the front line for the treatment

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of severe 2019-nCoV infections. Although the long-term use of these analogues in nonmalarial therapy such as rheumatoid arthritis or lupus demonstrates some severe adverse effects such as macular retinopathy and cardiomyopathy depending on the cumulative doses,[65,66] everyone concerns only the current world-wide life-threatening emergency situation. The adverse effects of CQ analogue therapy remain to be performed for 2019-nCoV-infected patients. However, CQ analogues are currently among the best existing drugs to impact the severity of 2019-nCoV infections in humans.

Antiviral mechanism action of chloroquine (CQ) analogues

Although the mechanism of action of CQ analogue against coronaviruses has not been completely elucidated, the increasing evidences suggest that the entry, replication and infection of several emerging viruses such as Ebola, Marburg, dengue, CHIKV, HIV etc. are highly dependent on autophagy process particularly autolysosomal stage or lysosomal acidification.[40,43] By alkalizing the autolysosome or neutrality of acidic pH in acidic vesicles like lysosomes, CQ analogue hampers the low-pH-dependent steps of these viral entry and replication, including fusion and uncoating into the cytoplasm of susceptible cells and thereby abrogate their infections.[25,44] Findings from previous studies have suggested that the analogue may inhibit the coronavirus through similar steps. The analogue can not only change the pH at the surface of cell membrane but also inhibit the autophagosome-lysosome fusion in autophagy process. The analogue also inhibits nucleic acid replication, glycosylation of viral proteins, virus assembly, new virus particle transport and virus release.[67]

Chloroquine (CQ) analogue as a late-stage autophagy disruptor

In eukaryotic cells, macroautophagy (hereafter called autophagy) is a genetically regulated evolutionary conserved catabolic process that facilitates nutrient recycling via lysosomal degradation of long-living unwanted cellular proteins as well as defective organelles, including endoplasmic reticulum.[68,69] Autophagy initiates with the formation of an isolation membrane (IM) through a series of chain reactions. By sequestrating with a recognized portion of intracellular components such as damaged organelles, this IM turns into a double-membrane vesicle called autophagosome which subsequently docks and fuses with a lysosome to become an autolysosome (AL). The contents of AL are degraded by the low pH and acidic enzymes such as proteases, lipases, nucleases, and glycases.[70,71] This multi-step complex process involves the engulfment of targets by autophagosomes and their subsequent degradation by lysosomes.[72] Lysosomal degradation is the last step in the process of autophagy, which generates molecules to be used for the synthesis of macromolecules (Figure 1a). Lysosomes, the garbage disposal system are intracellular organelles that harbored a plethora of soluble hydrolytic hydrolases capable of degrading macromolecules such as proteins, lipids, nucleic acids, polysaccharides, and cellular debris.[73,74] The healthy lysosome is segregated by a single lipid bilayer from the cytoplasm and housed in highly acidic lumen environment (pH of 4.5–5.0) relative to the slightly alkaline cytosol (pH 7.2)[74] in where these catabolic events occur. The acidic pH of lysosomes is critical for providing an optimal condition for its most hydrolytic enzymes to perform their catalytic activities, the movement and maturation of lysosomes as well as for vesicle fusion with other vacuolar compartments such as autophagosomes, a key step in autophagy. As a late-stage autophagy inhibitor (Figure 1a), CQ has been extensively used in cancer and viral research. Furthermore, CQ can inhibit autophagy by impairing autophagosome-to-lysosome fusion**(Figure 1a)** .[75]It is interesting to note that viral infection, especially Epstein-Barr virus (EBV), can activate constitutive autophagy to support virus latency.[76]EBV-induced autophagy is dependent on the expression of viral latent membrane proteins which provide cells with an improved survival ability. In these cells, viruses inhibit lysosomal degradation in the maturation step of autophagy and use autophagic membranes for the formation and release of the viral particles.[77] As a final stage autophagy inhibitor, CQ analogue is very important to protect the expression of viral latent membrane proteins as well as its survival ability in cells. As a negative regulator, mitogen-activated protein kinase (MAPK) regulates autophagy by two critical signalling pathways- mechanistic target of rapamycin (mTOR), a master regulator of autophagy -dependent and mTOR-independent pathways.[78] Thus, activation of cells via MAPK signalling pathway is frequently required by viruses to achieve their replication cycle.[79] CQ is known to inhibit phosphorylation (activation) of the p38 MAPK in THP-1 cells as well as caspase-1.[80] In the model of HCoV-229 coronavirus, CQ reduces cellular p38 MAPK activation and inhibits viral replication.[69] Thus, CQ may alter the

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2019-nCoV molecular crosstalk with its target cell by inhibiting MAPK pathway.

Chloroquine (CQ) analogue as an inhibitor of lysosomal acidification

CQ is a diprotic weak base. The structure of CQ contains a heterocyclic moiety (7chloro-quinoline) bearing a basic side chain at position 4 (Figure 1b). Two protonatable sites on the side chain are responsible for its slightly basic nature with pK's of 8.3 and 10.2, respectively, which makes it soluble in the stomach and thus orally administrable.[39,81] Structurally, HCQ differs from CQ by introducing a hydroxyl group at the end of the side chain. As a result of this structural modification, HCQ has ability to decrease to cross the blood-retinal barrier for retinal toxicity.[82-84] and more favourable safety profile than CQ.[85] The unprotonated form of CQ diffuses spontaneously and rapidly across the membranes of cells and organelles to acidic cytoplasmic vesicles such as endosomes, lysosomes or Golgi vesicles and thereby increases their pH.[39,86]Mechanistically, when CQ enters the acidic vesicles like lysosome, it becomes protonated and trapped due to the high internal acidity. In cellular levels, as a lysosomal lumen alkalinizer, the trapped CQ leads to decrease the activity of lysosomal and inactivate several lysosomal enzymes,[25,39,87] e.g., those required for proteolytic processing and post-translational modification of viral proteins (Figure 2a). Once oral administration, the analogue is readily absorbed and concentrated in tissues such as the lungs, liver, spleen and kidney-where several fatal viruses harboured, replicated and infected.[88] By increased acidic pH and/or structural changes in the Golgi apparatus with HCQ or by specific interaction with CQ, various enzymes e.g. glycosylating enzymes, glycosyltransferases are inactivated and glycosylation of SARS- coronaviruses is shown to suppress.[89,90] This event causes the structural changes in the gp120 glycoprotein and in turn reduces the reactivity and infectivity of newly produced virions.[91,92] A group of Chinese researchers reported that CQ was highly effective in reducing viral replication in Vero E6 cells infected by 2019-nCoV *in vitro*with an EC_{90} (effective concentration) 6.90 μM that can be easily achievable with standard dosing, due to its favourable penetration in tissues, including in the lung.[27] Here CQ blocks virus infection by increasing endosomal pH and by interfering with the glycosylation of cellular receptor of SARS CoV. CQ also interferes with 2019-nCoV attempts to acidify the lysosomes and presumably inhibits cathepsins, which require a low pH for optimal cleavage of 2019-nCoV spike protein.[93]

Chloroquine (CQ) analogue as an immunomodulator and anti-inflammatory agent

Lysosomes play roles in not only degradation of intracellular components, but also killing microbes in macrophages and dendritic cells through antigen processing and presentation.[48]Lysosomes remove the dead cell debris by resolution of inflammation and tissue remodelling.[94] The highly acidic lysosomal pH (<5) of scavenging M2-type macrophages are particularly effective in removing debris from tissues to help resolve inflammation; while M1 macrophages having lower lysosomal acidity (pH>5) show stimulation of immune responses. An M2-to-M1 macrophage transition can be experimentally achieved by CQ at least partially through raising lysosomal pH.[95] Moreover, CQ has the potential to alleviate pathological conditions associated with increased M2 activity such as vascular disorder during lung carcinogenesis.[96] Dendritic cells also restrict lysosomal acidification to optimize antigen processing and allow major histocompatibility complex (MHC) class-I dependent cross-presentation.[97]

By raising lysosomal pH and inhibiting lysosomal activity, CQ analogue particularly, HCQ can suppress antigen presenting cells (APCs), including plasmacytoid dendritic cells (pDCs) and B cells, and prevent antigen processing and MHC class II-mediated autoantigen presentation to T cells.[98] This event mediated by CQ analogue also suppresses T cell activation, differentiation and expression of co-stimulatory proteins such as CD154 on CD4+ T cells,[99] and cytokines formation T cells and B cells including interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNFα).[100] Beside affecting the virus maturation process, pH modulation by CQ can impair the proper maturation of viral protein,[101] and the recognition of viral antigen by dendritic cells, which occurs through a Toll-like receptor (TLR)-dependent pathway that requires endosomal acidification.[102] CQ analogue administration suppresses TLR signalling pathway by altering pH of endosomes and interrupting binding between TLR7 and TLR9 and their RNA/DNA ligands.[103-106] In the cytoplasm, the analogue also hinders the interaction between cytosolic DNA and the nucleic acid sensor **c** yclic **G** MP-**A** MP **s** ynthase (cGAS).[107] Since both TLR signalling and cGAS stimulation of interferon (IFN)

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genes (the STING pathway) are hindered by CQ analogue, consequent proinflammatory signalling activation and production of cytokines such as IFNs, IL-1 and TNF, are weakened (Figure 2b).[100] CQ also blocks TLR mediated activation of pDC and suppresses myeloid differentiation primary response gene 88 (MyD88) signalling by down-regulating the downstream signalling molecules, interleukin-1 receptor associated kinase 4 (IRAK-4) and IFN regulatory factor 7 (IRF-7).[108] Since TLR stimulation and production of IFN-α by pDC contribute to immune activation, blocking the pathway by using CQ analogues interfere emerging viral pathogenesis.[108] On the other hand, CQ can provide some conflicting effects on the immune system include increasing the export of soluble antigens into the cytosol of dendritic cells and the enhancement of human cytotoxic CD8+ T cell responses against viral antigens.[109]

Several studies have suggested that multiple organ failure and hypovolemic shock observed in fatal cases are most likely associated with the direct viral infection as well as the effects of proinflammatory cytokines, chemokines and other mediators released from infected and activated cells such as macrophages.[87,110-112] As an immunomodulatory agent, CQ analogue has also been beneficial in the treatment of viral infections and associated pathologies.[43,44] CQ analogue inhibits the production of several cytokines, chemokines or mediators, whose excessive appearance contributes the severity of viral infections. For example, one of the cytokines such as TNF-α is strongly implicated in filoviral pathologies which is able to activate macrophages. This cytokine causes to increase both the permeability and infectivity of endothelial cells.[110,113] It is suggested that CQ analogues are able to prevent the activation of macrophages and inhibit the secretion of TNF-α from various cells at clinically relevant concentration that can confer some benefits in the treatment of viral infections. [43,44] Another cytokine, IFN-γ has also been implicated in the pathologies of viral infections (e.g., Ebola). It has been reported that IFN-γ increase cellular sensitivity to apoptosis by up-regulating the expression of Fas and Fas ligand,[114] in fatal case of Ebola infection.[110] By inhibiting IFN-γ and TNF-α production and preventing the activation of macrophage,[110] CQ analogues appear a great therapy in the treatment of patients infected with Ebola. CQ is also found to inhibit IFNα, IFNβ, IFNγ, TNFα and IL-6 gene expression in U937 cells infected with dengue-2 virus *in vitro*. [47] As an adjuvant therapy, CQ analogues also regulate immune activation during HIV infection with other antiretroviral agents. The analogues are beneficial for chronic HIV-infected individuals because of reduction of systemic T-cell activation,[115-117] and immune hyperactivation in HIV/AIDS.[118]

It has been reported that the immune response of patients infected with 2019-nCoV virus results in the increase of cytokines IL-6 and IL-10.[15,119] This may progress to a cytokine storm, followed by multi-organ failure and potentially death. Here, CQ analogue can act indirectly through reduction of proinflammatory cytokine production in COVID-19 patients. In these settings, CQ analogue may be an ideal drug to treat 2019-nCoV infection as it can inhibit the viral mediated the cytokine storm. It is important to note that early treatment with these analogues may help to prevent the progression of the disease to a critical, life-threatening state. Hence, it is recommended that the concomitant use of low dose HCQ with an anti-inflammatory drug to help mitigate the cytokine storm in critically ill 2019-nCoV patients. However, the uses of corticosteroids or other immunosuppressants (e.g. tocilizumab) may be detrimental in critically ill 2019-nCoV infected patients because of prominent suppression of the immune system leading to an increased risk of superinfection.[120,121]

Chloroquine (CQ) analogue as a restrictor for binding of coronaviral receptors to target cells

Like other human coronaviruses, SARS CoV2 harbours three envelope proteins, the spike (S) protein (180–220 kDa), the membrane (M) protein (25–35 kDa) and the envelope (E) protein (10–12 kDa), which are required for entry of infectious virions into target cells. Using non-human coronavirus, it is shown that the M protein which localises to the trans-Golgi network, plays a vital role during viral assembly by interacting with the other proteins of the virus. Following assembly, the newly formed viral particles are transported to the cell surface in vesicles and are released by exocytosis.[41,122] CQ interferes with proteolytic processing of the M protein maturation,[25] and may alter virion assembly and budding of 2019-nCoV at the replication cycle.

It has also been reported that SARS-CoV-1 and MERS-CoV upregulate the angiotensin-converting enzyme

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2 (ACE2) expression in lungs, a process that can accelerate their replication and spread.[123]2019-nCoV is also found to utilise the same cell surface receptor ACE2 (expressed in lung, heart, kidney and intestine).[123,124] Binding to ACE2, 2019-nCoV can trigger conformational changes in the S glycoprotein allowing cleavage by the transmembrane protease TMPR-SS2 of the S protein and the release of S fragments into the cellular supernatant.[124,125,126] *In vitro* SARS-CoV-1 model, CQ has been shown to exert an attributable antiviral effect during pre- and post-infection conditions by interfering with the glycosylation of a viral cell surface receptor, ACE2 and blocking virus fusion with the host Vero cells.[90] This impaired terminal glycosylation of ACE2 may diminish the binding efficacy between ACE2 on target cells and the S protein of 2019-nCoV and consequently prevent infection.

A pH-dependant entry of coronavirus, SARS-CoV-1 into target cells is also mediated by S protein after binding of the DC-SIGN receptor.[127] The activation step that occurs in acidic endosomes results in fusion of the viral and endosomal membranes leading to the release of the viral SARS-CoV-1 genome into the cytosol.[128] In the absence of antiviral drug, the virus is targeted to the lysosome where lysosomal enzymes disrupt the viral particle and liberate the infectious nucleic acid for its replication.[129] CQ analogue can inhibit a pre-entry step of the viral cycle by interfering with viral particles binding to their cell surface receptor. The acidic monosaccharides, sialic acids found in cell transmembrane proteins are critical components of ligand recognition. CQ inhibits quinone reductase 2,[130] a structural neighbour of UDP-N-acetylglucosamine 2-epimerases,[131] that are involved in the biosynthesis of sialic acids. The possible interference of CQ with sialic acid biosynthesis can account for the broad antiviral spectrum against HCoV-O43 because of sialic acid moieties as its receptors.[132] Although the binding of SARS-CoV to sialic acids has not been reported so far, if 2019-nCoV like other coronaviruses targets sialic acids on some cell subtypes, this interaction may also be affected by CQ treatment.[133,134]

Clinical trials of chloroquine (CQ) analogues against COVID-19

A recent study suggests that CQ is found to block COVID-19 infection at low-micromolar concentration, with a half-maximal effective concentration (EC_{50}) of 1.13 μM and a half-cytotoxic concentration (CC_{50}) greater than 100 μM after a 48-hour incubation time *in vitro*. [27] Recently, *In vitro* five FDA-approved drugs and two broad spectrum antivirals have been evaluated against a clinical isolate of 2019-nCoV.[27] The above report concludes that "CQ is highly effective in the control of 2019-nCoV infection *in vitro* " and its "safety track record suggests that it should be assessed in human patients suffering from the novel coronavirus disease". Thus, several clinical trials are currently investigating the use of CQ analogues particularly HCQ to treat 2019-nCoV infection. In addition, recently an open label non-RCT study of twenty COVID-19 cases indicates that the combination of HCQ (600mg daily) and azithromycin is significantly associated with viral load reduction/disappearance in COVID-19 patients compared to controls. The addition of azithromycin to the HCQ combination causes significantly more efficient for virus elimination.[135] However, it is important to note that the dosing regimens used in these trials are mainly based on previous clinical experience, raising the alarm that adverse effects may occur in study participants. In these studies, an optimized dosing regimen is designed as high loading dose and low maintenance dose HCQ based on its unique pharmacokinetics (i.e. high accumulation in cells and long elimination half-life).

Another recent publication also suggests that CQ phosphate is superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus negative conversion, and shortening the disease course.[29] Severe adverse reactions to CQ phosphate are not noted in the aforementioned patients.[29] These clinical trials are conducted in different hospitals and possibly followed a number of different clinical protocols among those listed in Table 3. These trials also include various designs for control groups (none, different antivirals, placebo, etc.) and various outcome primary indicators. The final interpretation is therefore technically demanding, and in the absence of published data, it is difficult to reach any firm conclusion. It is also the utmost importance to know if the observed efficacy is associated specifically with CQ phosphate, or if this includes other salts (e.g., sulfate) of CQ, and HCQ. It is also necessary to determine if the benefit of CQ therapy depends on the Age and sex class, the clinical presentation or the stage of the disease.

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Other potential repurposing candidates for the treatment of COVID-19

Immediate therapeutic options in response to the 2019-nCoV outburst are urgently needed. So, a number of repurposing available candidates have joined the list of antiviral agents that can be used as therapeutic arsenal against COVID-19.

Virally targeted agents

FDA approved nucleoside analogues such as favipiravir (T-705) and ribavirin and experimental nucleoside analogues including remdesivir (GS-5734) may have potential against 2019-nCoV. Favipiravir approved for influenza treatment can effectively inhibit the RNA-dependent RNA polymerase of RNA viruses such as influenza, Ebola and chikungunya.[136] A recent study suggests its activity against 2019-nCoV at EC_{50} = 61.88 μM in Vero E6 cells.[27] Thus, patients with 2019-nCoV are being recruited in randomized clinical trials (RCT) to evaluate the efficacy of favipiravir plus interferon-α (ChiCTR2000029600) and favipiravir plus baloxavir marboxil, an approved influenza inhibitor (ChiCTR2000029544).[23] Ribavirin approved for treating hepatitis C virus (HCV) and respiratory syncytial virus (RSV), has been evaluated in patients with SARS and MERS, but its side effects including anaemia may be prominent at high doses,[137] and its sufficient potency against 2019-nCoV is uncertain. Remdesivir has broad-spectrum activities against RNA viruses such as MERS and SARS in cell cultures and animal models, and has also been tested in a clinical trial for Ebola.[23] A recent study indicates that remdesivir inhibits 2019-nCoV at EC_{50} = 0.77 μM in Vero E6 cells.[27] Two phase III trials (NCT04252664 and NCT04257656) have already been initiated in early February to evaluate remdesivir in patients with 2019-nCoV. Other FDA-approved drugs such as penciclovir, nafamostat, lopinavir and ritonavir have also been evaluated for the antiviral efficiency. Protease inhibitors such as lopinavir and ritonavir have been reported to be active against SARS and MERS and their clinical trials (e.g., ChiCTR2000029539) have been initiated to test the efficacies in patients infected with 2019-nCoV.

Host-targeted agents

Teicoplanin, a glycopeptide antibiotic routinely used to treat*Staphylococcal* infections, has showed efficacy against various viruses such as Ebola, influenza, HIV, and on coronaviruses such as MERS-CoV and SARS-CoV.[138,139] In coronaviruses, teicoplanin acts on the early step of the viral life cycle in human cells by inhibiting the low pH cleavage of the viral S protein by cathepsin L in the late endosomes thereby preventing viral replication cycle.[138] This activity is conserved on SARS CoV-2 as the target spike S protein sequence that serve as cleavage site for cathepsin L,[140] and the concentration of teicoplanin required to inhibit 50% of viruses (IC_{50}) is 1.66 μM*in vitro* .[141] Pegylated interferon α-2a and -2b, approved for the treatment of hepatitis B virus (HBV) and HCV, can be used to stimulate innate antiviral responses in patients infected with 2019-nCoV, and trials involving interferons have been initiated, such as a combination of pegylated interferon plus ribavirin (ChiCTR2000029387). However, it is uncertain whether this combination could act synergistically against 2019-nCoV.[23] Nitazoxanide, approved for diarrhoea treatment, can also inhibit 2019 nCoV at EC_{50} = 2.12 μM in Vero E6 cells.[27] The antiviral efficacy of such agents needs to be assessed in clinical studies.

Conclusions, recommendations and future perspective

CQ analogue particularly HCQ is considered to be safe and a cheap drug, and its side-effects are generally mild and transitory. As a result, it has been used for more than 100 years. CQ approved by FDA for malaria treatment, has long been prescribed prophylactically to pregnant women at risk of exposure to *Plasmodium* parasites.[142]As an anti-inflammatory agent, these analogues are efficacious for the treatment of rheumatoid arthritis and systemic lupus erythematosus.[39] HCQ is found to be more potent than CQ at inhibiting 2019-nCoV *in vitro* . Thus, CQ analogue reportedly exhibits antiviral activities with *in vitro* efficacy against several viruses, including coronaviruses;[143] however, real time data concerning its *in vivo* efficacy during viral infection and anti-CoV activity in living animals remains limited. In light of the possibility of using CQ to fight orphan viral infections,[67] and the urgent clinical demand, CQ analogue shows potentially favourable risk-benefit balance, that is the relatively high safety, and the low expenditure of such treatment in the context

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of the current COVID-19 outbreak.[144] There is sufficient pre-clinical rationale and evidence regarding the effectiveness of CQ analogue for treatment of COVID-19 as well as evidence of safety from long-time use in clinical practice for other indications.[41] Since the current COVID-19 outbreak cases were reported in more than 85 countries so far, the low cost of CQ is a major benefit for both the highly stressed healthcare systems of involved high-income countries and the underfunded heath care systems of middle- and low-income counties.[145] Thus, CQ analogue may become a real breakthrough as broad-spectrum anti-coronaviral agents to treat COVID-19 for future epidemics.

The margin of safety between the therapeutic and toxic dose is narrow and CQ poisoning has been associated with cardiovascular disorders and retinopathy that can be life-threatening.[146]Therefore, these analogue uses should be subjected to strict rules, and self-treatment is not recommended. The vital ethical issue is administration of CQ analogue in the setting of COVID-19 is experimental as declared by WHO, and therefore it requires ethical trial approval, or off-label (i.e. ethically justifiable as the best available treatment). As even off-label use of CQ, it may be accompanied by several concerns; the first priority is patient safety. Such use should be accompanied by close monitoring. In case of epidemic situation, it is hardly the ideal setting to do this. Moreover, the ethical approach to off-label drug use also differs between countries.[24] Thus, based on*in vitro* evidence and still unpublished clinical experience, the expert panel recommended CQ phosphate tablet, at a dose of 500 mg twice per day for 10 days, for patients diagnosed as mild, moderate and severe cases of 2019-nCoV pneumonia, provided that there were no contraindications to the drug.[18,147] The panel recommends using several precautions, including blood testing to rule out the development of anaemia, thrombocytopenia or leukopenia as well as serum electrolyte disturbances and/or hepatic and renal function dysfunction. Other recommendations are routine electrocardiography to rule out the development of QT interval prolongation or bradycardia and patient interviews to seek the appearance of visual and/or mental disturbance/deterioration. The Dutch Centre of Disease control (CDC) suggested to treat severe infections requiring admission to the hospital and oxygen therapy or admitted to the ICU with CQ.[24,148]

Although the use of CQ analogue may be supported by expert opinion, clinical use of this drug in patients with COVID-19 should adhere to the Monitored Emergency Use of Unregistered Interventions Framework (MEURI) framework or after ethical approval as a trial as stated by the WHO. Data from high-quality, coordinated, clinical trials coming from different locations worldwide are urgently needed. The worldwide ongoing clinical trials will verify whether the hopes raised by CQ in the treatment of COVID-19 can be confirmed. The rapid identification of effective interventions against 2019-nCoV is a major challenge. Given the available knowledge on their safety profiles, and in some cases efficacy against closely related coronaviruses, repurposing existing antiviral agents is a potentially important near-term strategy to tackle 2019-nCoV. With the ongoing efforts to prevent the spread of 2019-nCoV worldwide, we hope that the outbreak may subside in a few months, as with SARS and MERS. Nevertheless, the outbreak has emphasized the urgent need for renewed efforts to develop broad-spectrum antiviral agents to combat coronaviruses.

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Table 1: Key unanticipated events in the history of repurposing CQ analogues[a]

Year	Discovery/ events
Before 1532	Indigenous Quina-quina bark is applied in South America to treat febrile illness
1632	Quina-quina bark is used to treat for "tertian fever" in Peru
1629-1633	The Romantic legend of Countess of Chinchon is cured with quina-quina bark
1633	Jesuit priest Bernabe' de Cobo transported the bark from Peru to Europe (Spain)
1600-1700	Quina-quina powder is distributed throughout Europe and Asia for febrile illness
1742	Quina-quina tree is renamed as Cinchona tree by Carolus Linnaeus (botanist)
1818	Quinine is isolated from cinchona tree bark for the treatment of malaria
1894	Quinine is the first prescribed form to treat lupus by Dr. J.F. Payne
1908	Nuclear structure of quinoline is indispensable for antimalarial activity.
1920	The first synthetic antimalarial drug, pamaquine
1930	Quinacrine is developed to treat malaria
1931	Ehrlich group synthesized quinacrine
1934	Hans Andersag synthesized resochin
1939	Resochin is renamed as chloroquine
1940	Quinacrine is used in Russia for lupus
World War II	The improvement of inflammatory diseases in British soldiers by quinacrine
1945	HCQ is synthesized. Clinical trials in USA approved for human use
1946	FDA-approved CQ for treatment of malaria
1951	Effectiveness of quinacrine in the treatment of lupus
1955	FDA-approved Plaquenil (HCQ sulfate) for SLE and CLE treatment.
1956	Improvement of inflammation in RA patients by CQ
1959	Triquin (HCQ, CQ and QC combination) is FDA-approved to treat lupus
1960	Anticancer properties of CQ
1970	As lysosomotropic agent, CQ is first shown to inhibit cell growth of tumour *in vitro.*
1970	Banned clioquinol in response to controversy association with SMON in Japan
1972	FDA-approved for Triquin withdrawn and is pulled off the market
1974	CQ withdrawn from Japanese market claim as SMON and retinopathy
1980-90	CQ analogs are investigated as autophagy inhibitors *in vitro*
1989	The first reflection that CQ has an anti-cancer effect in Burkitt's lymphoma
1998	The first observation that CQ is an autophagy inhibitor

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Year	Discovery/ events
2000	Anticancer properties of HCQ
2003	First clinical trial of CQ for the antitumour effects of CQ in glioblastoma.
2007	The synergistic effect CQ in combination with anticancer drugs
2009	Debut of HCQ in Japan for clinical care
2010-	CQ analogs in emerging viral infectious diseases (AIDS, SARS, Dengue)
2014-	HCQ in clinical trials for selectively targeted autophagy in cancer patients
2017	HCQ overcome anticancer drug resistances
2019-2020	HCQ is used for the treatment of COVID-19

Abbreviations: SLE: systemic lupus erythematosus; CLE: cutaneous lupus erythematosus; SMON: subacute myelo-optic neuropathy. QC: quinacrine.

[a]Major references [39,88,89]

Table 2: Activities of chloroquine analogues against coronaviruses

Agents; Reference	Targeted virus	System used for antiviral activity screening	Antiviral effect
CQ[46]	SARS-CoV	Vero (African green monkey kidney) E6 cells	EC_{50} = 8.8±1.2 μM
CQ[111]	SARS-CoV	Vero E6 cells	EC_{50} = 4.4±1.0 μM
CQ, CQ-P, CQ-2P[30]	SARS-CoV (four strains)	Vero 76 cells	CQ: EC_{50} = 1-4 μM CQ-P: EC_{50} = 4-6 μM CQ-2P: EC_{50} = 3-4 μM
		BALB/c mice	IP or IN CQ, starting 4 h prior to virus exposure: 50 mg/kg but not 10 mg/kg or 1 mg/kg reduced for the IN route (but not the IP route) viral lung titres from mean ± S.D. of 5.4 ± 0.5 to 4.4 ± 1.2 in $\log_{10}$ $CCID_{50}$/g at Day 3
CQ, HCQ[30]	SARS-CoV	Vero cells	CQ: EC_{50} = 6.5 ± 3.2 μM HCQ: EC_{50} = 34 ± 5 μM
	Feline coronavirus	CRFK cells	CQ: EC_{50} > 0.8 μM HCQ: EC_{50} = 28 ± 27 μM
CQ[76]	HCoV-229E	Human epithelial lung cells (L132)	CQ at 10 μM and 25 μM inhibited HCoV-229E release into the culture supernatant

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Agents; Reference	Targeted virus	System used for antiviral activity screening	Antiviral effect
CQ[69]	HCoV-OC43	HRT-18 cells New-born C57BL/6 mice; CQ administration TP and via maternal milk	EC_{50} = 0.306 ± 0.0091 μM 100%, 93%, 33% and 0% survival rate of pups when mother mice were treated with 15, 5, 1 and 0 mg/kg/day body weight, separately
CQ[30]	FIPV	*Felis catus* whole fetus-4 cells	Inhibition of FIPV replication by CQ in concentration dependent
CQ[78]	SARS-CoV MERS-CoV HCoV-229E-GFP	Vero E6 cells Huh7 cells Huh7 cells	EC 50 = 4.1 ± 1.0 μM EC 50 = 3.0 ± 1.1 μM EC 50 = 3.3 ± 1.2 μM
CQ[41]	SARS-CoV-2	Vero E6 cells	EC 50 = 1.13 μM

Abbreviations: CQ-P: Chloroquine monophosphate; CQ-2P: Chloroquine diphosphate; IP: Intraperitoneal; IN: Intranasal; TP: transplacentally; $CCID_{50}$: 50% cell culture infectious dose; CoV: coronavirus; EC_{50}: 50% effective concentration; GFP: Green fluorescent protein; HCoV: human coronavirus; MERS: Middle East respiratory syndrome; SARS: Severe acute respiratory syndrome; FIPV: Feline infectious peritonitis virus; CRFK: Crandell–Reese feline kidney; HCoV-229E-GFP: (GFP-expressing recombinant HCoV-229E).

Table 3: Selected examples of clinical trials of chloroquine analogues in patients with COVID-19 in China[a]

Trial ID	Study design	Intervention	Comparison group(s)	COVID-19 (n patients)	Primary outcomes
Recruiting status	*Recruiting status*	*Recruiting status*	*Recruiting status*	*Recruiting status*	*Recruiting status*
ChiCTR2000030031	Single Centre; RCT	CQ-P; BID	Placebo; BID	Mild and common pneumonia (n = 120)	Time of conversion to be negative of novel coronavirus nucleic acid
ChiCTR2000029988	Single Centre; RCT	CQ-P	Standard treatment	Severe pneumonia (n = 80)	Time to clinical recovery
ChiCTR2000029899	Single Centre; RCT	HCQ: Day 1: 1st dose: 6 Tab (0.1 g/Tab), 2nd dose: 6 Tab (0.1 g/Tab) after 6 h; Day 2–10: 2 Tab/day (0.1 g/Tab)	CQ-P: Day 1–3: 500 mg, BID, Day 4–10: 250 mg BID	Mild and common Pneumonia (n = 100)	Time to clinical recovery

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Trial ID	Study design	Intervention	Comparison group(s)	COVID-19 (n patients)	Primary outcomes
ChiCTR2000029898	Single Centre; RCT	HCQ; Day1: 1st dose: 6 Tab (0.1 g/Tab), 2nd dose: 6 Tab (0.1g/Tab) after 6 h; Day 2˜10: 2 Tab/day (0.1g/Tab)	CQ-P; Day 1–3: 500 mg BID; Day 4–10: 250 mg BID	Severe pneumonia (n = 100)	Time to clinical improvement
ChiCTR2000029939	Single Centre; RCT	CQ-P	Standard treatment	Pneumonia (n = 100)	Length of hospital stay
ChiCTR2000029935	Single Centre	CQ-P	No comparison group	Pneumonia (n = 100)	Length of hospital stay
ChiCTR2000029868	Multi-Centre; RCT	Oral HCQ-S Tab	Standard treatment	Pneumonia (n = 200)	Viral nucleic acid test
ChiCTR2000029761	Multi-Centre; RCT	Low-dose HCQ group Medium-dose HCQ group; High-dose HCQ group	Standard treatment	Common pneumonia (n = 240)	Negative conversion rate of COVID-19 nucleic acid; lung inflammation absorption ratio
ChiCTR2000029741	Multi-Centre; RCT	CQ-P	Lopinavir/Ritonavir	Mild and common Pneumonia (n = 112)	All-cause mortality at day 28; length of stay
ChiCTR2000029740	Single Centre; RCT	Oral HCQ; 0.2g BID	Standard treatment	Pneumonia (n = 78)	Negative conversion rate of COVID-19 nucleic acid
ChiCTR2000029542	Single Centre; PCS	Oral CQ; 0.5 g BID for 10 days	Standard treatment	Pneumonia (n = 20)	Negative conversion rate of COVID-19 nucleic acid; 30-day cause specific mortality
ChiCTR2000029559	Single Centre; RCT	Group 1: oral HCQ; 0.1 g BID; Group 2: oral HCQ; 0.2 g BID	Placebo: Oral starch pill BID	Pneumonia (n = 300)	Negative conversion rate of COVID-19 nucleic acid; T cell recovery time
ChiCTR2000029762	Single Centre; RCT	HCQ Tab	Standard treatment	Pneumonia (n = 60)	Negative conversion rate of COVID-19 nucleic acid; lung inflammation absorption ratio

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Trial ID	Study design	Intervention	Comparison group(s)	COVID-19 (n patients)	Primary outcomes
Pending approval status	*Pending approval status*	*Pending approval status*	*Pending approval status*	*Pending approval status*	*Pending approval status*
ChiCTR2000029609	Multi-Centre; Non-RCT	Mild-moderate CQ group: oral CQ-P; Mild-moderate combination group: CQ-P plus Lop/Rit; Severe CQ group: oral CQ-P	Mild-moderate Lop/Rit group: oral Lop/Rit; Severe Lop/Rit group: oral Lop/Rit	Pneumonia (n = 205)	Negative conversion rate of COVID-19 nucleic acid
ChiCTR2000030054	Single Centre RCT	HCQ-S group: HCQ-S 0.2 g BID x 14 days; CQ-P group: 1st dose of CQ-P 1 g x 2 days, then 0.5 g x 12 days	Standard treatment	Mild and common pneumonia (n = 100)	Clinical recovery time
ChiCTR2000029992	Single Centre RCT	CQ-P group: CQ-P 1.0 g x 2 days, then 0.5 g x 12 day from the third day HCQ-S group: HCQ-S 0.2 g BID x 14 days	Standard treatment	Severe pneumonia (n = 100)	Clinical recovery time; Changes in viral load of whole respiratory tract samples compared with the baseline
ChiCTR2000029975	Single Centre; Single-arm	150 mg CQ-P in 5 ml of normal saline, inhaled by atomization for one week	No comparison group	Pneumonia (n = 10)	Viral negative-transforming time; Time from severe and critical patients to clinical improvement
ChiCTR2000029803	Single Centre; RCT	Group A1: HCQ, small dose; Group A2: HCQ, high dose	Group B1: Arb-HCl low dose; Group B2: Arb-HCl high dose	Positive test of COVID-19 nucleic acid (n = 320)	Progression to suspected or confirmed disease within 24 days
ChiCTR2000029826	Single Centre; RCT	2 Tab CQ-P; BID	2 Tab placebo; BID	Critically ill pneumonia (n = 45)	Mortality rate
ChiCTR2000029837	Single Centre; RCT	2 Tab CQ-P; BID	2 Tab placebo; BID	Mild and common Pneumonia (n = 120)	Negative conversion rate of COVID-19 nucleic acid

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Trial ID	Study design	Intervention	Comparison group(s)	COVID-19 (n patients)	Primary outcomes
Not yet recruiting status	*Not yet recruiting status*	*Not yet recruiting status*	*Not yet recruiting status*	*Not yet recruiting status*	*Not yet recruiting status*
ChiCTR2000030417	Single Centre; RCT	CQ-P aerosolized inhalation	WFI atomized inhale combined	Pneumonia (n = 30)	Temperature normal for more than 3 days, respiratory symptoms
NCT04261517	Single Centre; RCT	HCQ 400 mg/day for 5 days	Standard treatment	Pneumonia (n = 30)	Mortality rate at day 14
NCT04286503	Multi-Centre; RCT	Carrimycin	Lop/Rit; or Arb or CQ-P	Critically ill pneumonia (n = 520)	Fever to normal time; pulmonary inflammation resolution time at 30 day

Abbreviations: BID: twice per day; RCT: Randomized controlled trial; PCS: Prospective cohort study; CQ-P: Chloroquine phosphate; HCQ: Hydroxychloroquine; HCQ-S: Hydroxychloroquine sulphate; Lop/Rit: Lopinavir/ritonavir; Arb-HCl: Arbidol hydrochloride; Tab: Tablets; 1st : First; 2nd : Second; WFI: Water for injection.

[a]Adapted from[24]

Figure legends:

Figure 1. An overview of inhibition of autophagy process and repurposing CQ analogue development

Figure 1a: Inhibition of autophagy by CQ

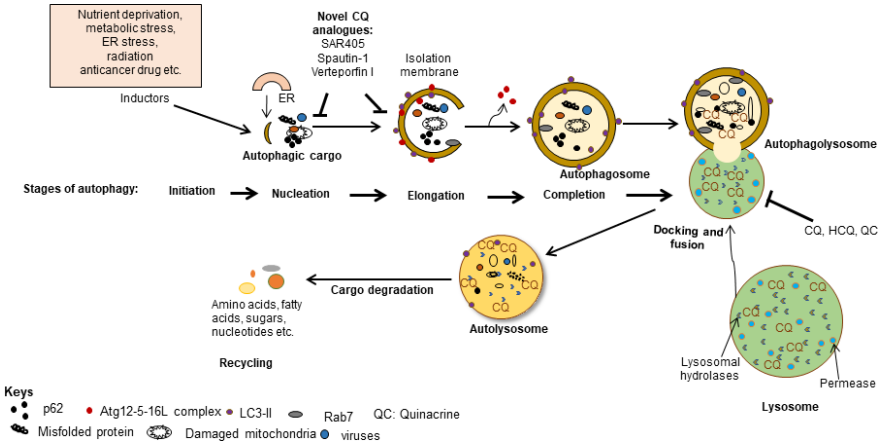

Figure 1b. Repurposing CQ analogue development

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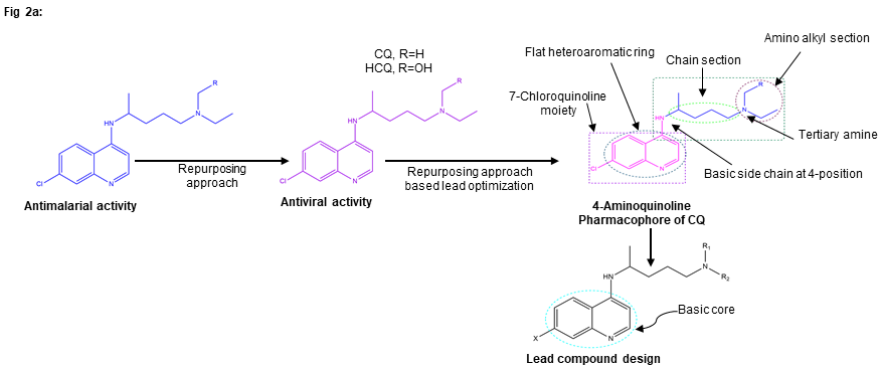

Figure 2. Lysosomotrophic and immunomodulating effects of CQ analogue on SARS-CoV-2 replication cycle

Figure 2a. Lysosomotrophic effect of CQ

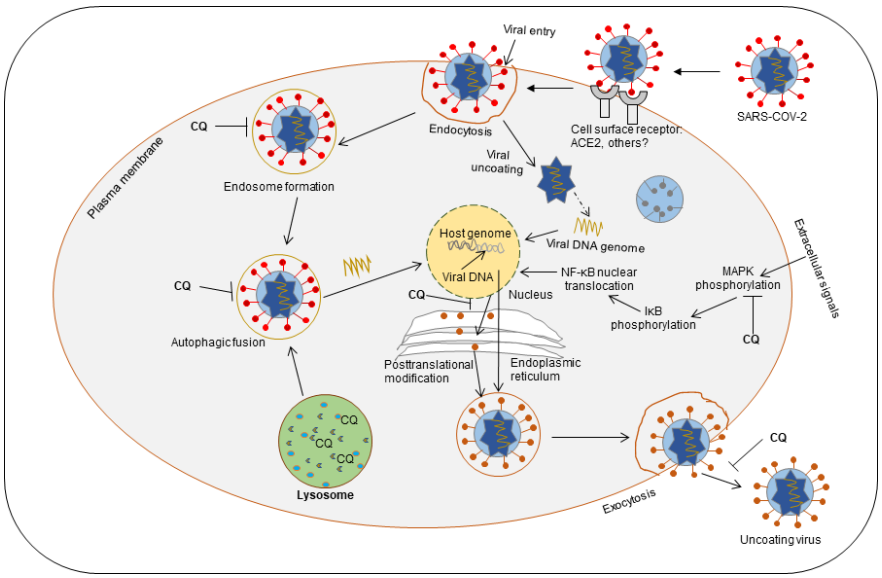

Figure 2b. immunomodulating effects of CQ

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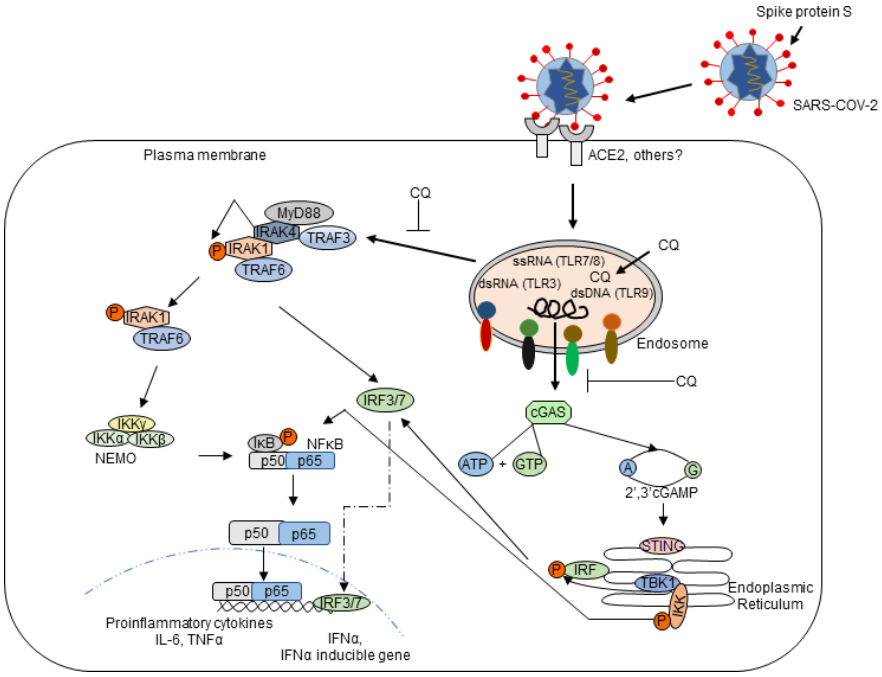

CQ becomes highly concentrated in such acidic organelles such as lysosome leading to dysfunction of several enzymes, e.g. those required for proteolytic processing and post-translational modification of viral proteins. CQ inhibits both TLR signalling and cGAS stimulation of interferon (IFN) genes (STING) pathway. Reverse transcription intermediates from CoV is recognized by cGAS, which catalyzes the production of cGAMP to bind and activate the ER-resident adaptor protein STING. STING then forms a complex with TBK1 and translocates from the ER to the perinuclear lysosomal compartments via an autophagy-like process. The STING–TBK1 complex subsequently activates transcription factors IRF3 and NF-ϰB to induce the production of type I IFNs and inflammatory cytokines to establish an antiviral state. cGAMP: cyclic GMP-AMP; cGAS: cyclic GMP-AMP synthase; IFN: interferon; IRF3: interferon regulatory factor 3; TRAF3: TNF receptor–associated receptor 3; TRAF6:TNF receptor–associated receptor 6; NF-ϰB: nuclear factor-ϰB; P: phosphorylation; STING: stimulator of interferon genes; TBK1: TANK binding kinase 1.

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