

Chloroquine as a Potential Treatment and Prevention Measure for the 2019 Novel Coronavirus: A Review

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ABSTRACT

There is a long trail of research studies testing the in vitro and in vivo efficacy of chloroquine and its derivatives in treating and preventing infection by various coronavirus species. More recent findings have highlighted the possibility of treating patients infected with the 2019 novel coronavirus, SARS-CoV-2. This review describes the mechanism of coronavirus infection, the mechanism of action of chloroquine, and summarizes the available literature highlighting the efficacy of chloroquine as an anti-coronavirus agent. These findings should encourage the wider scientific community to conduct thorough research on the possible efficacy of chloroquine and its derivatives in treating and preventing SARS-CoV-2 infection.

KEYWORDS

Antimalarial; Chemoprophylaxis; Chloroquine; Coronavirus; COVID-19; Global Health;

Hydroxychloroquine; Public Health; SARS-CoV-2; Virus

INTRODUCTION

In December of 2019, a novel human coronavirus outbreak started in Wuhan, Hubei Province, China and then subsequently spread to dozens of other countries becoming a global pandemic.¹ Severe-acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was found to be the viral agent responsible for COVID-19 pneumonia, the resulting illness from infection. As of this writing, there are 169,387 cases and 6,513 deaths globally,² although the actual rates maybe higher considering low testing and case identification in some regions. The elderly and those who have underlying comorbidities have been disproportionately been afflicted.^{3,4} This outbreak has additionally seen a high morbidity among healthcare workers,² much like previous outbreaks of human coronavirus.⁶⁻⁸ The high morbidity among healthcare workers is likely due to both the close contact with infected individuals in a hospital setting as well as evidence of subclinical transmission of the disease.⁹ Alarmingly, despite best efforts to protect healthcare workers, there have also been several deaths among health providers fighting the epidemic.⁵ The burden of disease placed on healthcare workers is problematic for a multitude of reasons including the costs associated with caring for and quarantining infected individuals, the spread of disease in a clinical setting to vulnerable patients, and the strain it places on response efforts due to incapacitated healthcare workers. Preventive measures and effective therapeutics to protect healthcare workers and those who are at risk are vital to combat an infectious disease epidemic. Presently, there are no commonly agreed on prophylactic or therapeutic treatment options for SARS-CoV-2 and a vaccine is not expected to be developed and disbursed to the wider population for 12-18 months.¹⁰ However, there are a number of clinical trials ongoing to study the efficacy of older drugs to be repurposed for use against SARS-CoV-2. One such medication includes the antimalarial chloroquine (CQ), which was recently cited as a potential treatment to

shorten SARS-CoV-2 disease course, mitigate inflammatory responses to infection, inhibit the exacerbation of pneumonia, improve lung imaging findings, and promote a virus negative conversion.^{3,11,12} A number of clinical trials are currently underway as listed on the Chinese Clinical Trial Register to study the efficacy of CQ and its derivatives to treat SARS-CoV-2 (ChiCTR2000029939, ChiCTR2000029935, ChiCTR2000029899, ChiCTR2000029898, ChiCTR2000029868, ChiCTR2000029837, ChiCTR2000029826, ChiCTR2000029803, ChiCTR2000029762, ChiCTR2000029761, ChiCTR2000029760, ChiCTR2000029741, ChiCTR2000029740, ChiCTR2000029609, ChiCTR2000029559, ChiCTR2000029542).¹³ It is worth noting that CQ is recommended to be included in the next version of the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 issued by the National Health Commission of China.^{11,14} On February 19, 2020, the National Health Committee of the People's Republic of China issued the "Diagnosis and Treatment of New Coronavirus Pneumonia (Trial Version 6)," which included the addition of chloroquine-phosphate as an antiviral treatment.¹⁵ Multinational and Chinese drug-makers soon thereafter sped up the manufacturing and supply of chloroquine phosphate and the pharmaceutical company Bayer has expedited the supply and transportation of Resochin (chloroquine phosphate) to Guangdong provincial government at the heart of the outbreak.¹⁶ Furthermore, in South Korea, the Korea Biomedical Review has also included the use of of the CQ derivative hydroxychloroquine (as CQ is not available in the country) for the treatment of those infected with SARS-CoV-2.¹⁷

Given the time-consuming nature of developing, testing, and producing new therapeutics, the repurposing of existing pharmaceuticals offers a pragmatic alternative in responding quickly and efficiently to novel diseases. With CQ in particular, there is ample experience in using this drug

in clinical and population-level settings, its required dosing in humans, its safety and absorption, distribution, metabolism, and excretion. What follows is a review of the mechanisms of coronavirus infection as well as the mechanism of CQ that serve as evidence of its possible efficacy. In addition, this review explores the available literature on the efficacy of CQ and its derivatives for various coronavirus infections *in vitro* and *in vivo*. References used in this review were identified through a search of PubMed with the terms “Chloroquine” and “Coronavirus.” Articles resulting from this search and relevant references were reviewed and included here. Only articles published in English were reviewed. This review aims to highlight the possible efficacy of CQ as a possible agent to both prevent infection in healthcare providers and vulnerable populations, as well as to treat those infected with the virus.

Mechanism of Action of Coronavirus Infection

Coronaviruses (CoVs) are enveloped, plus-strand RNA viruses belonging to the family Coronaviridae in the order Nidovirales.¹⁸ SARS-CoV-2 has many common features with the coronavirus family³ and has a phylogenetic similarity to the previous species of SARS-CoV-1 from the outbreak from 2002-2003.¹⁹ More recent studies found SARS-CoV-2 mimicked SARS-CoV-1 in its severity.^{3,20,21} Coronavirus populations exhibit considerable genetic heterogeneity,²² however there are common underlying mechanisms.

Coronavirus delivery of virus particles into the host cell requires binding of the virus to cellular receptors followed by a clathrin-mediated endocytosis to create a viral endosome.^{18,22,23} This process is mediated by a viral surface glycoprotein termed Spike, a homotrimer of S proteins,²⁴ binding to the type I integral membrane receptor angiotensin-converting enzyme-2 (ACE2),²⁵ followed by a pH-independent endocytotic reaction. It is worth noting that ACE2 is expressed at

high levels in type I and II alveolar cells in the lungs.^{26,27} Once internalized, only then does the fusion of virus with lysosomes depend on a low endosomal and lysosomal pH.²⁸ The S-glycoprotein is cleaved into S1 and S2 subunits by endosomal proteases cathepsin B and L, with the resulting S2 subunit mediating membrane fusion.²⁹ Cathepsin B and L activity are inhibited by an elevated endosomal pH. Viral entry into the cytoplasm is likewise dependent on an acidic endosomal pH.³⁰ Once released into the cytosol, the virus utilizes a viral RNA-dependent RNA-polymerase (i.e. Replicase) to drive the viral replication,³¹ create virions for exocytosis, and thus further the infection of neighboring cells. Previous studies on Human Coronavirus (HCoV-HKU1) indicate that infection of alveolar cells is associated with the surface expression of viral Spike protein, mediating membrane fusion with neighboring cells leading to syncytium formation.³² This allows direct cell to cell spread of virus which could play a role in the pathogenesis of lung disease and immune system evasion. However, these mechanisms may proceed differently in the novel SARS-CoV-2 as pathogenesis has been shown to differ between coronaviruses.³³⁻³⁶

Common symptoms of COVID-19 illness include fever, cough, fatigue, muscle pain, dyspnea (i.e. shortness of breath), expectoration, headaches, hemoptysis (blood in the saliva), and diarrhea.²⁰ Laboratory tests revealed elevated levels of liver enzymes, depleted white blood cell counts, and elevated heart enzymes indicating cardiac impairment. The majority of patients given chest computerized tomography (CT) scans were found to have bilateral ground glass-like opacities and subsegmental areas of consolidation²⁰ indicative of COVID-19 induced pneumonia.³⁷ Severe forms of disease are associated with progression to Acute Respiratory Distress Syndrome (ARDS)³⁸ and septic shock.²⁴ In some patients, immune response to the virus resulted in an increase in inflammatory cytokines,^{20,39} which may progress to a “cytokine storm,”

followed by multi-organ system dysfunction. For severe cases of disease, more invasive life-saving measures are indicated including admission to the ICU, mechanical ventilation, and extracorporeal membrane oxygenation (ECMO).⁴⁰ There is a higher preponderance of severe disease in patients of older age and who have underlying co-morbidities such as cardiovascular disease, diabetes, chronic respiratory disease, and oncological diseases.^{24,40} Despite life-saving measures, the case fatality rate (CFR) is calculated to be anywhere between 0.5% and 5.0%,^{3,24,40-42} although the actual CFR may differ significantly as the screening and identification of positive cases differs greatly from country to country.⁴³

Mechanism of Action of Chloroquine

Chloroquine is a 4-aminoquinoline discovered in 1934 and has predominantly been used to prevent and treat malaria.⁴⁴ In addition, it has been used as an anti-inflammatory agent for the treatment of a number of diseases.^{11,45} CQ and its derivatives act as weak bases, which preferentially accumulate within intracellular compartments (including endosomes, lysosomes, and Golgi vesicles) causing a number of downstream effects; in particular, a relative increase in the endosomal and lysosomal pH,⁴⁵ although the mechanism of action of CQ remains under continuous study in modern molecular medicine.^{46,47}

CQ and its derivative hydroxychloroquine (HCQ) have characteristic wide volume of distribution and both can distribute to aqueous cellular and intercellular compartments resulting in long mean residence times (~900 hours for CQ and ~1,300 hours for HCQ).⁴⁵ CQ has a half-life of around 50-days and has a high renal clearance, which is an important clinical consideration in patients with kidney failure as decreased clearance increases the bioavailability of CQ. The most common adverse effect of these antimalarial drugs are gastrointestinal effects

including nausea, vomiting, diarrhea, and abdominal discomfort.⁴⁸ An important consideration is that several studies have reported the incidence of cardiotoxic effects, including rhythm disorders (such as a prolonged QT interval) and the development of cardiomyopathy in patients with rheumatic diseases,⁴⁹⁻⁵² but conclusive evidence is lacking and further pharmacovigilance is required. The most severe complication attributed to antimalarial treatment is the development of retinopathy with prolonged use as these drugs can cause retinal damage by disrupting an important step in the visual cycle mediated by lysosomal degradation.⁵³ Retinopathy is more commonly associated with CQ than with HCQ and can result in patients developing retinal defects including circular and diametric defects.

When added extracellularly, the non-protonated portion of CQ enters the cell, where it becomes protonated and concentrated in acidic, low-pH organelles.²⁵ By increasing the pH of endosomal compartments,⁵⁴ CQ might impair the maturation of lysosomes and autophagosomes and inhibit antigen presentation along the lysosomal pathway.⁵⁵⁻⁵⁷ Interference of lysosomal activity might have an immunomodulatory effect as it could impair antigen presentation via the lysosomal pathway. Lysosomes contain hydrolytic enzymes and work with other vesicles to digest intra- and extracellular material which is an underlying mechanism for antigen processing and MHC class II presentation, indirectly promoting immune activation.⁵⁸ CQ and HCQ have a good safety profile and are generally considered to function as immunomodulatory rather than immunosuppressant drugs. They have not been associated with an increased risk of infectious complications⁵⁹ or cancer.⁶⁰

RESULTS

Chloroquine as an Anti-Coronavirus Agent

There is compelling evidence from early trials in China highlighting the efficacy of CQ and HCQ in the treatment of SARS-CoV-2.^{11,61,62} In addition, there is a long trail of studies that have investigated the efficacy of CQ and its analogues, HCQ and ferrocene-chloroquine (FQ), with various species of coronavirus (Table 1). The findings, summarized and presented here, highlight the possibility that CQ and its derivatives may be effective against the novel SARS-CoV-2, which shares a close phylogenetic ancestry with previous species of coronavirus. CQ has multiple activities, one of which is to increase the pH of intracellular vesicles as mentioned earlier, which interferes with the pH-dependent steps of viral replication, including fusion and uncoating.⁶³ It is believed that lysosomotropic agents (of which CQ is included) can effectively inhibit coronavirus as the virus requires acidification of endosomes for proper functioning.^{33,44} For enveloped viruses, post-translational modification of the envelope glycoproteins occurs within the endoplasmic and trans-Golgi network vesicles. This process involves proteases and glycosyl-transferases, some of which require a low pH. CQ-induced altered ACE2 glycosylation is also believed to be the mechanism by which treatment of coronavirus infected individuals acts by stopping S-protein binding and subsequent phagocytosis²⁵ and release into the cytoplasm where viral replication occurs.¹⁸ Besides its direct antiviral effect, CQ suppresses the production of cytokines which could mediate the inflammatory complications of viral diseases.⁴⁴

Severe Acute Respiratory Syndrome (SARS) Coronavirus-1/2

Both SARS-CoV-1 and -2 were studied in various cell culture lines *in vitro* (see Table 1). More recently, Wang et al. and Yao et al. tested the efficacy of CQ and HCQ in Vero cells derived from the kidney of an African green monkey. Wang et al. conducted time-of-addition assays studying the efficacy of a number of potential compounds, including CQ and the broad-spectrum antiviral nucleotide analog, Remdesivir.⁶¹ The authors demonstrated that CQ functioned at both

pre- and post-entry stages of infection, demonstrating CQs antiviral activity *in vitro*. Yao et al. studied the efficacy of CQ and HCQ in Vero 6 cells. The authors additionally built a physiologically-based pharmacokinetic (PBPK) model to predict drug concentrations in human tissues. These models have been developed to predict the exposure, response, and time course of a drug for different dosage regimens in a target population.⁶⁴ The study authors found both CQ and HCQ to have antiviral activity *in vitro* and decreased viral replication in a concentration-dependent manner.⁶²

In vitro studies have also been conducted on SARS-CoV-1, which has many commonalities, and has a close phylogenetic relationship with, the novel SARS-CoV-2 belonging to the same Subgroup B lineage of betacoronaviruses.¹⁹ The efficacy of CQ against SARS-CoV-1 was assessed by both Vincent et al. and Keyaerts et al. Vincent et al. infected Vero E6 cells and treated them with both CQ and NH₄Cl, another weak base known to alter intracellular pH.²⁵ The study authors found that with pre-treatment, there was a concentration-dependent decrease in virally infected cells, with the complete elimination of infection *in vitro* at higher concentrations. These findings were reproduced for post-infection treatment experiments, although higher concentrations of CQ were required to inhibit the spread of infection. Interestingly, the authors also studied the effect of CQ on ACE2 receptors and viral S-protein production. Although they discovered that the surface expression of ACE2 or the level of processed S-protein did not change with treatment, they did find a decrease in the terminal glycosylation of ACE2, which may serve to render the ACE2-SARS-CoV-1 interaction less efficient. Keyaerts et al. had similar results in their earlier study.⁶⁵ Biot et al. prepared new CQ and HCQ derivatives, ferroquine (FQ) and hydroxyferroquine (HFQ), which retained antimalarial activity against *P. falciparum*.⁶⁶ The authors subsequently tested the efficacy of these new compounds against SARS-CoV-1 and

feline coronavirus and discovered these new compounds were able to exhibit inhibition of SARS-CoV-1 replication with high selectivity. It is worth noting that HCQ was ineffective against both feline coronavirus and SARS-CoV-1. de Wilde et al. likewise screened a large library of FDA approved drugs against several coronavirus species including SARS-CoV-1, as well as MERS-CoV and Human-CoV-229E *in vitro*. Of the screened compounds, CQ was found among several other candidates to effectively inhibit the *in vitro* growth of SARS-CoV-1 with no cytotoxicity.

Middle-East Respiratory Syndrome (MERS) Coronavirus

MERS-CoV is also classified under the same betacoronavirus genus as SARS-CoV-1/2, but belongs to Subgroup C lineage.⁶⁷ MERS coronavirus infection occurs via a similar mechanistic pathway as SARS-CoV-1/2, with the exception that it enters the cell via the dipeptidyl peptidase 4 (DPP4) receptor instead of ACE2.⁶⁸ *In vitro* studies of CQ efficacy for MERS-CoV infection were conducted with mixed results (see Table 1). Cong et al. tested the efficacy of several candidate compounds (including CQ) in human monocyte-derived macrophages (MDMs) and immature monocyte-derived dendritic cells (MDDCs).⁶⁹ Study authors revealed that CQ did not show any detectable antiviral activity in MDMs or MDDCs, although little or no cytotoxicity was reported. However, de Wilde et al. mentioned before, additionally found CQ to be an effective compound in inhibiting MERS-CoV replication *in vitro* among other coronaviruses.⁷⁰

Other Species of Coronavirus (MHV4, Human CoV-OC43, Human CoV-229E, PHEV, FIPV)

Other species of coronavirus were studied *in vitro* and *in vivo* and the efficacy of CQ treatment were verified (see Table 1). CQ treatment was studied with two species of coronaviruses often

responsible for the common cold during the winter and early spring, Human CoV-OC43 and Human Cov-229E.⁷¹ de Wilde et al. found CQ to act as a broad-spectrum antiviral against several coronavirus species, including HCoV-229E, a coronavirus belonging to the alphacoronavirus genus.⁷⁰ Kono et al. additionally tested the efficacy of CQ to elucidate its mechanism of action against HCoV-229E and discovered that the drug had a neutralizing effect on intracellular signaling proteins needed for viral replication, but had no effect on the virus prior to internalization.⁷² CQ efficacy was also tested against the Human CoV-OC43 species, another coronavirus belonging to Subgroup A lineage of betacoronaviruses. Interestingly, Keyaerts et al. and Niu et al. studied the efficacy of CQ treatment *in vivo* in mouse models. For their mouse study, Keyaerts et al. passed on CQ from maternal mice either transplacentally or via maternal milk to newborn pups.⁷³ The authors noted a 100% survival rate for newborn mice who were inoculated with HCoV-OC43 but whose mothers were pre-treated with high dose CQ injections prepartum, with the survival rate dropping off in a dose-dependent manner. The overall survival rate of newborn mice treated with CQ via maternal milk was found to be 69% at the highest dose of CQ given, with a decreasing survival rate with lower doses. *In vitro* experiments were also conducted by Keyaerts in human cell lines with varying concentrations of CQ as well time-of-addition assays. The study determined that CQ inhibits the *in vitro* replication of HCoV-OC43 with high selectivity, however, time-of-addition assays showed CQ is required at the moment of infection, with a loss of antiviral activity when CQ is added at later time points. Niu et al. confirmed that prophylactic CQ treatment acted to prevent virus replication in mice challenged with HCoV-OC43.⁷⁴ CQ efficacy, along with its derivatives, were tested in multiple other species of coronavirus in various models. Biot et al. found that CQ and its derivative compounds, FQ and HFQ, were able

to inhibit replication of feline coronavirus *in vitro* with high selectivity.⁶⁶ However, HCQ was surprisingly ineffective in controlling both SARS-CoV-1 and feline coronavirus replication. Ding et al. explored the mechanism of autophagy induction in mouse neural cells inoculated with Porcine hemagglutinating encephalomyelitis virus (PHEV), a betacoronavirus belonging to the Subgroup A lineage.²⁸ They then tested various compounds and their ability to control PHEV replication, including CQ. The authors found PHEV infection leads to atypical autophagy, however, they revealed that CQ was able to suppress PHEV infection. Gallagher et al. studied the infection of a selected mouse cell line by the betacoronavirus Mouse Hepatitis Virus Type 4 (Subgroup A lineage) to elucidate the mechanism of infection.²² The study authors found that MHV4 inoculated cells treated with endosomotropic weak bases, including CQ, in the presence of persistent infection did not stop virus entrance into cells, but CQ effectively eliminated viral RNA synthesis, replication, and the processing of viral glycoprotein S. Lastly, Takano et al. studied the efficacy of CQ in both *in vitro* and *in vivo* in the presence of Feline Infectious Peritonitis Virus (FIPV), an alphacoronavirus.⁷⁵ For FIPV infected cats treated with varying schedules of CQ, viral mRNA expression was lower in the pre-treatment group, the clinical score of CQ-treated groups were better than for CQ-untreated groups, and the CQ pre-treated group additionally had the highest relative survival at 34.3 days post infection.

CONSIDERATIONS

Although its side-effect profile is low, CQ does have some documented adverse effects. Many patients taking CQ have reported gastrointestinal upset, headache, blurred vision, insomnia, and QT interval prolongation.⁷⁶ Another well-known adverse effect is the development of retinopathy with prolonged use.⁴⁵ CQ and HCQ have few contraindications, however patients should avoid

use who have underlying retinopathy or a history of hypersensitivity to this class of compounds. CQ acts as an immunomodulatory agent which promotes apoptosis in cells, a critical tool of the hosts immune system in fighting infection. High CQ use globally has been theorized to lead to the emergence of diseases which have developed anti-apoptotic mechanisms, including HIV.⁷⁷ CQ may not be sufficient to stop viral infection, but used in conjunction with other antivirals, it may be an effective means of treatment.⁶¹ Lastly, the mechanism of action of CQ and all of its downstream effects are not fully understood. Recent findings by Xue et al. have unearthed that CQ also acts as an ionophore that aids in the intracellular transport of the mineral Zinc.⁷⁸ This is an important consideration and this mechanism should be explored further given that Zinc salts have been found to block the replication of Hepatitis E virus by inhibiting the activity of viral RNA-dependent RNA-polymerase, a molecule used by SARS-CoV-2 and other coronaviruses for viral replication.⁷⁹

CONCLUSIONS

The prospect of having an effective treatment for the protection of vulnerable populations and healthcare workers as well as for the treatment of those infected is noteworthy. CQ is already widely available as an anti-malarial, well tolerated, cheap, easy to take, has a low toxicity profile, and has limited contraindications for use. There is ample evidence of the *in vitro* and *in vivo* efficacy of CQ and its derivatives in the prevention and treatment of multiple species of coronavirus, including *in vitro* experiments with SARS-CoV-2.^{61,62} There are a number of clinical trials that are currently underway to test CQ and HCQ against SARS-CoV-2 as mentioned earlier. Considering its safety has already been scrutinized and it has been widely prescribed for use as an anti-malarial, Phase III randomized double-blind placebo controlled

studied are need to assess the efficacy of CQ as a chemoprophylactic and treatment measure for SARS-CoV-2. Preliminary findings from Wang et al. have highlighted this drug's efficacy in treating SARS-CoV-2 infected individuals,¹¹ but the trial itself has not been completed as of this writing and trial data has not been disseminated for further scrutiny. CQ and its derivatives show early promise to treat SARS-CoV-2 and should be explored as a potential preventive and therapeutic measure to turn the tide of this rapidly growing pandemic.

FUNDING

No funding was or sponsorship was received for this study or publication of this article.

ACKNOWLEDGMENTS

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

DISCLOSURES

John E. Kearney declares that he has no conflict of interest.

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Table 1: Studies showing the *in vitro/in vivo* efficacy of CQ (and its derivatives) against coronavirus species

Author	Year	CQ or CQ Analog	Coronavirus Species	Genus	Lineage	Study Design	Results
Wang et al.	2020	CQ (w/ Remdesivir)	SARS-CoV-2	Betacoronavirus	Subgroup B	Standard assays were carried out to measure the effects of several compounds, including CQ and the anti-HIV-1 drug Remdesivir. Vero E6 cells* were used to assess cytotoxicity and infection in the presence of varying concentrations of test drugs.	Time-of-addition assays demonstrated that CQ functioned at both entry and at post-entry stages of SARS-CoV-2 infection in Vero E6 cells.
Yao et al.	2020	CQ, HCQ	SARS-CoV-2	Betacoronavirus	Subgroup B	Pharmacological activity of CQ and HCQ were tested using SARS-CoV-2 infected Vero Cells.* The authors also built a physiologically-based pharmacokinetic (PBPK) model to predict drug concentrations in human tissues.	Results showed that both drugs had antiviral activity <i>in vitro</i> and were found to decrease viral replication in a concentration-dependent manner. HCQ exhibited a superior <i>in vitro</i> antiviral effect.
Vincent et al.	2005	CQ	SARS-CoV-1	Betacoronavirus	Subgroup B	Vero E6 cells* were infected and treated with CQ and ammonium chloride pre- and post-infection. In addition, CQ activity against SARS-CoV-1 was studied by characterizing the drug's effect on cell surface expression of ACE2, structural changes to the ACE2 receptor, and the biosynthesis and processing of SARS-CoV-1 spike protein.	Authors found a concentration-dependent decrease in virus antigen-positive cells, with the complete abolishment of SARS-CoV-1 infection at higher concentrations in pre-treatment experiments. The results were replicated with post-infection treatment, with higher concentrations of CQ almost completely inhibiting the spread of infection to adjacent cells in culture. The authors found that CQ did not alter the surface expression of ACE2 receptors, but found that there was a decrease in the terminal glycosylation of ACE2, which is thought to render the ACE2-SARS-CoV-1 interaction less efficient and inhibit viral entry. Last, the authors found that, except for higher concentrations, there was no decrease in biosynthesis and in levels of processed virus spike glycoprotein.
Biot et al.	2006	FQ, HFQ (Ferroquine, Hydroxy-ferroquine)	SARS-CoV-1, feline coronavirus**	Betacoronavirus	Subgroup B	Authors prepared a new class of CQ derivatives (HF/HFQ) which closely mimic the activity of antimalarial drugs. HFQ was tested to see if it could retain antimalarial activity against <i>P. falciparum</i> and a different set of viruses including SARS-CoV-1 and feline coronavirus.	FQ was found to be effective inhibitors of SARS-CoV-1 replication in Vero cells. FQ and HFQ compounds were also found to inhibit feline coronavirus <i>in vitro</i> . These compounds also exhibited a high selectivity index as well. CQ was used as a control and found to inhibit both SARS-CoV-1 and feline coronavirus, while HCQ did not.
Keyaerts et al.	2004	CQ	SARS-CoV-1	Betacoronavirus	Subgroup B	Authors tested the efficacy and cytotoxicity of CQ against SARS-CoV-1 in Vero E6 cells.* Authors also conducted time-of-addition assays to test the efficacy of CQ at time of infection as well as 8 hours post-infection.	In the virus yield assay, no significant infection was observed <i>in vitro</i> 1- and 3-days post-infection. It was found that a higher concentration of CQ was needed 3-days post-infection to inhibit viral replication.
de Wilde et al.	2014	CQ	SARS-CoV-1, MERS-CoV, Human CoV-229E	Betacoronavirus (SARS-CoV-1, MERS-CoV), Alphacoronavirus (H-CoV-229E)	Subgroup B (SARS-CoV-1) Subgroup C (MERS-CoV)	Screening for FDA-approved compounds with anti-MERS-CoV activity. A primary library screen was performed using a set of 348 FDA-approved drugs that were evaluated for their ability to inhibit the replication of MERS-CoV in Vero cells.	Authors found that CQ inhibits MERS-CoV and Human-CoV-229E replication <i>in vitro</i> at low-micromolar concentrations in the absence of cytotoxicity of uninfected control cells. In addition, these molecules appear to be broad-spectrum coronavirus inhibitors, as they blocked the replication of human coronavirus 229E and SARS-CoV-1 with comparable efficacy.

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Author	Year	CQ or CQ Analog	Coronavirus Species	Genus	Lineage	Study Design	Results
Cong et al.	2018	CQ	MERS-CoV	Betacoronavirus	Subgroup C	Authors tested the efficacy of several candidate MERS-antivirals (including CQ) in human monocyte-derived macrophages (MDMs) and immature monocyte-derived dendritic cells (MDDCs). Cytotoxicity assays were performed in parallel to measure drug toxicity. Antiviral activity was evaluated by means of a 50% tissue culture infectious dose.	CQ did not show any detectable antiviral activity in MDMs or MDDCs, although little to no cytotoxicity was associated with the compound.
Keyaerts et al.	2009	CQ	Human CoV-OC43	Betacoronavirus	Subgroup A	<p>Authors tested various concentrations of CQ against the human coronavirus-OC43 in a human cell line (HRT-18). Time-of-addition assays were also conducted where CQ was added at the time of infection or at different time points thereafter.</p> <p>Mouse infection studies were also conducted where one population of female mice were treated with CQ which was passed onto newborn pups transplacentally who were subsequently inoculated with virus (HCoV-OC43). Another population of newborn pups were exposed to CQ via maternal milk of CQ injected maternal mice. The pups were then inoculated with HCoV-OC43.</p>	<p>CQ inhibits the <i>in vitro</i> replication of HCoV-OC43 with a high selectivity.</p> <p>For time-of-addition assays, measurements of viral load show that CQ is required at the moment of infection to block HCoV-OC43 replication. At later time points, a loss of the antiviral activity of CQ was noted.</p> <p>For newborn mice studies, authors found that all pups untreated died within 6 days post-HCoV-OC43 challenge. A 100% survival rate was found in litters of the mother mice that were treated with high concentrations of CQ prepartum, with decreasing survival rates in a dose-dependent manner. The survival rate of newborn mice that received CQ via maternal milk was 69%.</p>
Niu et al.	2008	CQ	Human CoV-OC43	Betacoronavirus	Subgroup A	Authors tested the efficacy of CQ treatment <i>in vivo</i> in a mouse model. Mice were treated prophylactically with CQ and then infected intranasally with HCoV-OC43.	Mice treated with CQ exhibited no detectable signal from the virus in bioluminescent assays. Western blot analysis of infected mice confirmed the efficacy of CQ in preventing virus replication.
Kono et al.	2008	CQ	Human CoV-229E	Alphacoronavirus		Authors studied the correlation between CQ and the activation of intracellular signaling protein production in HCoV-229E infected human fetal lung cells, the effect of CQ on viral replication, and the effect of CQ on the internalization of virus.	CQ treatment was demonstrated to have a neutralizing effect on intracellular signaling proteins needed for viral replication. CQ has no influence on the process prior to the internalization of HCoV-229E.
Ding et al.	2017	CQ	PHEV (Porcine hemagglutinating encephalomyelitis virus)	Betacoronavirus	Subgroup A	Authors explored the mechanism of autophagy induction by PHEV infection in Neuro-2a cells, and the role of autophagy in PHEV replication. The authors also investigated the change in viral replication by various compounds including CQ.	PHEV infection was found to induce atypical autophagy which is involved in viral replication. After infection with PHEV, treatment with CQ suppressed PHEV infection.
Gallagher et al.	1991	CQ	MHV4 (Coronavirus Mouse Hepatitis Virus Type 4) ***	Betacoronavirus	Subgroup A	Selected mouse cells were used for propagation of wild-type MHV4 in persistently infected cells. Virus-specific RNA synthesis was monitored. Variants of the mouse line that were persistently infected with MHV4 were selected for by the formation of extensive syncytium.	Authors found that the weak base CQ does not inhibit viral fusion and endocytosis but it did inhibit internal processing of the virus needed for cytosolic release and replication as well as processing of glycoprotein S (spike) for further infection.

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Author	Year	CQ or CQ Analog	Coronavirus Species	Genus	Lineage	Study Design	Results
Takano et al.	2013	CQ	FIPV (Feline Infectious Peritonitis Virus)	Alphacoronavirus		<p>Authors investigated if CQ inhibited FIPV replication in a feline fetal cell line.</p> <p>CQ efficacy was also studied <i>in vivo</i> in FIPV infected cats in a time-of-addition assay.</p>	<p>FIPV replication was inhibited in a CQ dose-dependent manner in both pre- and post-treatment groups in infected feline cells.</p> <p>For FIPV infected cats treated with varying schedules of CQ, viral mRNA expression was lower in the pre-treatment group. The clinical score of CQ-treated groups were better than for CQ-untreated groups. The CQ pre-treated group additionally had the highest relative survival.</p>

* Isolated from the kidney epithelial cells of African Green Monkey.

** Feline coronavirus in Crandell-Reese feline kidney (CRFK) cells.

*** This species of coronavirus was selected for as it is dependent on endosomal pH for phagocytosis.