Pharmacological Advances of Chloroquine and Hydroxychloroquine: From Antimalarials to Investigative Therapies in COVID-19

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Abstract
During the coronavirus disease 2019 (COVID-19) pandemic, numerous existing chemicals have been screened for antiviral potential against the emerging coronavirus severe acute respiratory syndrome coronavirus 2. Chloroquine and hydroxychloroquine, after exhibiting potent in vitro efficacy, have gained tremendous attention. Both therapeutics are derivatives of natural alkaloid quinine and were first synthesized to treat malaria. Thereafter, the pharmaceutical applications of the agents have expanded to many new areas. In this article, the medicinal history and pharmacological activities of chloroquine and hydroxychloroquine are summarized. Antimalarial, anti-inflammatory, antitumor, antiviral properties, and therapeutic potential in the emerging viral infection COVID-19 are discussed. Pharmacokinetics, adverse effects, and toxicities are reviewed.

Keywords
chloroquine, hydroxychloroquine, covid-19, broad-spectrum antiviral, antitumor, clinical pharmacology

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Overview
Chloroquine (CQ) and hydroxychloroquine (HCQ) are both synthetic therapeutics first developed to mimic natural alkaloid quinine for its antimalarial effect.1 For centuries, the bark of Cinchona trees (Cinchona spp., Rubiaceae) has been used to treat malaria and fevers.2 The therapeutic application of this natural product originated in South America, before making its way to European countries. It was then realized that quinine and other alkaloids extracted from cinchona bark are responsible for the antimalarial properties. Quinine subsequently became the standard medicinal prophylaxis and treatment for malaria.2 To mask its bitter taste, people started mixing quinine with sugar and gin,3 hence the creation of the popular modern drink gin and tonic. In World War II, Europeans lost access to the major Cinchona tree plantation in Java.4 With the advancement of medicinal chemistry, scientists soon synthesized quinine-like chemicals including CQ and HCQ to continuously combat malaria.1,4 Over the past decades, an array of therapeutics structurally derived from quinine has been developed. Clinical applications of the class have greatly diversified. Quinidine, for instance, has shown therapeutic effects for arrhythmia and Alzheimer’s disease.5 Chloroquine (CQ) and hydroxychloroquine (HCQ) 2 of the most commonly used quinine derivatives, have been constantly studied and utilized for more disease states beyond malaria.

Pharmacological Activities of CQ and HCQ

Antimalarial Activity
CQ (C18H26ClN3) was first successfully synthesized in 1934. HCQ (C18H26ClN3O) was developed in 1946 as a less toxic therapeutic alternative (Figure 1).6,7 At first, both were primarily indicated for malaria as quinine derivatives. Their role in malaria treatment has changed over the past decades due to increasing resistance.2

Quinine derivatives, including CQ and HCQ, are weak bases. They accumulate in acidic food vacuoles of intraerythrocytic trophozoites. The antimalarial mechanism of action is to induce selective toxicity to lysosomes, thereby preventing hemoglobin degradation.9

However, the evolving mutations in transporters PfCRT and PfMDR1 impair the entry of CQ to the digestive vacuole. Energy-coupled efflux of CQ is also observed to decrease the accumulation of CQ.10 Nowadays, CQ is rarely used against...
Plasmodium falciparum due to widespread resistance. CQ remains as an effective therapy for malaria caused by Plasmodium ovale and Plasmodium malariae as well as Plasmodium vivax in most regions. HCQ can be used as a substitute therapy for CQ-sensitive malaria. HCQ is ineffective in CQ-resistant Plasmodium strains.

**Anti-Inflammatory Activity**

The use of quinines for anti-inflammatory purposes has been a subject of study since the 1890s. Presently, HCQ is the more commonly used of the 4-AQs as a disease-modifying antirheumatic drug in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Both drugs continue to be evaluated for additional inflammatory conditions, including Sjogren's Syndrome, antiphospholipid syndrome, and inflammatory bowel disorders (IBD). As weakly basic compounds, CQ and HCQ enter the acidic lysosomal environment and disrupt lysosomal enzymatic activities. Specifically, they inhibit autophagy and prevent the activation of major histocompatibility complex class II-mediated antigens. CQ and HCQ also increase endosomal pH, which is believed to inhibit Toll-like receptors involved in cytokine production pathways. Additional pathways are still being explored, including ligand binding interference of cyclic guanosine monophosphate-adenosine monophosphate synthase and the reduction of antiphospholipid antibodies (aPL)."}

The efficacy of CQ and HCQ may depend on the disease state, as shown in in vitro studies. One study in antiphospholipid syndrome found that HCQ at 10 μM fully blocked the effects of interleukin-1β (IL-1β) and aPL on IL-8 but did not completely block tumor necrosis factor-α (TNFα) in murine cells or in monocytes. An in vitro study in patients with RA and SLE indicates that HCQ at a higher concentration (100 μM) significantly inhibits IL-6, IL-17, and IL-22 in monocytes. A mouse model PK study of IBD synthesized a nondegradable polymeric CQ (pCQ). The research showed that 7 doses of pCQ and HCQ at 30 mg/kg HCQ equivalent every other day reduced colon inflammation (P ≤ 0.05) and proinflammatory activity to varying degrees in IL-6 (P ≤ 0.01), IL-2 (P ≤ 0.05), and IL-1β (P = not significant [NS]). Of note, the synthetic pCQ (half-maximal inhibitory concentration [IC₅₀] >2000 μg/mL) showed lower cytotoxicity against HepG2 cell line than HCQ (IC₅₀ = 42 μg/mL). Though used in a broad range of anti-inflammatory conditions, having larger-scale human trials testing specific targets for action will illuminate the best uses for CQ and HCQ.

**Antitumor Activity**

Starting in 1970, CQ and HCQ have been the subject of numerous oncology studies to treat cancers such as brain cancer, colorectal cancer, pancreatic cancer, renal cancer, and chronic myeloid leukemia. They have been studied the most in glioblastoma (GBM), colon cancer, and pancreatic cancer. Due to side effects at high doses of monotherapy, CQ or HCQ have been mainly trialed for synergistic effects with other antitumor agents in several forms of cancer. Presently, the most recognized antineoplastic mechanism of action for both CQ and HCQ is via lysosomal autophagic inhibition. As weakly basic compounds, CQ and HCQ gain proton ions within the acidic lysosomal environments of tumors, disrupting the enzymatic degradation chain of autophagy. It has also been demonstrated that CQ affects autophagy by impairing the fusion of autophagosomes to lysosomes. One study recently discovered that CQ and HCQ target palmitoyl-protein thioesterase 1, resulting in lysosomal mTORC1 displacement and autophagy. Contrastingly, CQ and HCQ's autophagic effects have also led to...
tumor promotion in earlier stages of tumor development; further studies will aid in discerning where CQ and HCQ can consistently target autophagy to ensure tumor-suppressant effects.22,25

GBM Phase I, II, and III trials are elucidating when to optimally utilize CQ or HCQ in conjunction with other chemotherapies. A study of GBM cell lines found that the combination of HCQ (5 μg/mL) with bevacizumab (100 μg/mL) inhibited tumor cell proliferation and that HCQ increased cell sensitivity to bevacizumab.26 A murine test of quinoline-based drugs showed that CQ and HCQ (25 mg/kg) induce apoptosis, autophagy, and endoplasmic reticulum stress in temozolomide (TMZ)-resistant GBM cells.27 A 3 + 3 phase I study (N = 16) looked at HCQ 200-800 mg/day with TMZ (75 mg/m2) and found HCQ 600 mg/day to be the maximum tolerated dose, as all 3 subjects taking 800 mg daily experienced Grade III/IV myelosuppression. Thereafter, the noncomparative phase II portion of the study (N = 76) utilized a 2-compartment PK model for HCQ and demonstrated that HCQ's dose-proportional exposure yielded inconsistent autophagy inhibition; multiple patients experienced grade III/IV adverse events with potential or definite relationships to HCQ or TMZ.28

When given with carmustine (200 mg/m2), CQ (150 mg/day) portion of the study (N = 61) utilized a 2-compartment PK model for HCQ and demonstrated that HCQ's dose-proportional exposure yielded inconsistent autophagy inhibition; multiple patients experienced grade III/IV adverse events with potential or definite relationships to HCQ or TMZ.28

In a Phase I study, 9 elderly patients with symptomatic pancreatic cancer received CQ 100, 200, and 300 mg per week, timed to be given 1 day after gemcitabine treatment. Three patients in the CQ 100 and 200 mg per week groups showed tumor regression, which is not observed in the 300 mg weekly group.29 In contrast, a Phase I/II study (N = 35) with pancreatic adenocarcinoma showed that HCQ taken at escalating doses (from 200/ mg up to 1200 mg/day for 31 days) with gemcitabine showed no improvement in observable clinical outcomes. However, this study also measured changes in the HCQ autophagy marker LC3; the patients with >51% increase in LC3 had better survival, suggesting a future opportunity to identify patients who will respond to autophagy prior to treatment selection.30

**Antiviral Activity—Broad Spectrum and in COVID-19**

As early as in the 1940s, quinine was studied in mice for potential efficacy against influenza virus.31 Since then, quinine and its derivatives have been evaluated for various viral infections.32 In the 1960s, CQ was researched in the mouse hepatitis virus and encephalomyocardiitis virus.33,34 Since then, CQ and HCQ have been tested against Zika, Ebola, human immunodeficiency virus, hepatitis C virus, coronaviruses, and others,35,36,37 exhibiting broad-spectrum antiviral properties.

The mechanism of action for CQ / HCQ as antivirals is not fully understood. It appears to involve multiple pathways and is not identical in all viruses. A well-known broad-spectrum mechanism is to neutralize acidic endosomal pH, therefore blocking endosome-mediated viral entry.14,42 CQ and HCQ also exhibit anti-inflammatory and immunomodulatory benefits in viral infections. They inhibit the production and release of cytokines, including IL-1, 2, 6, 18, TNF-α, and interferon-gamma (IFN-γ).43,44 Among research for the ongoing coronavirus disease 2019 (COVID-19) pandemic, a recent in-silico test shows that CQ and HCQ can compromise the binding affinity of S protein on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to gangliosides on the host cell surface.45

During the SARS outbreak, CQ was found to inhibit SARS-CoV in vitro, showing potential as both prophylactic and therapeutic treatment.46 Since the COVID-19 outbreak, CQ has shown a potent antiviral effect on SARS-CoV-2 in vitro, with the 90% effective concentration (EC90) of 6.90 μM, which is clinically achievable.47 SARS-CoV-2 in vitro studies have also revealed achievable effective concentrations of HCQ, with 1 study finding HCQ (EC50 = 0.72 μM) to be more potent than chloroquine (EC50 = 5.47 μM)48 and another study finding CQ (EC50 = 2.71, 3.81, 7.14, and 7.36 μM) more potent than HCQ (EC50 = 4.51, 4.06, 17.31, and 12.96 μM).49 An open-label nonrandomized clinical trial (N = 42) in France studied the HCQ regimen of 200 mg 3 times a day orally with and without azithromycin (AZM). The study reported 100% viral clearance.
on day 6 in the HCQ + AZM group versus 57.1% in the HCQ group versus 12.5% in the control group.\textsuperscript{47} Notably, the HCQ group had a higher viral load at baseline. While the viral suppression data are promising, clinical outcomes were not studied. A follow-up study was conducted by the French team on the same HCQ + AZM regimen (N = 80).\textsuperscript{48} Clinical outcomes were evaluated this time. Time from treatment initiation to discharge was 4.1 ± 2.2 days. End results included 15% (n = 12) utilization of oxygen therapy, 3.8% (n = 3) transfers to intensive care unit, and 1.2% (n = 1) mortality. This was an observational study without a control group.

A controlled pilot study in Shanghai presented HCQ as ineffective for expediting viral clearance, recovery from fever, and computed tomography image improvement (P > 0.05).\textsuperscript{49} However, the study had a very small sample size (N = 30). It is not a treatment versus a placebo study. Both HCQ group and control group were treated with interferon (IFN), and most patients also received antiviral lopinavir/ritonavir or arbidol. The study design is questionable, especially when there are no definite conclusions on the effect of IFN and antivirals in COVID-19 yet.\textsuperscript{50} In April 2020, a retrospective multicenter study (N = 368) in American veterans with COVID-19 showed HCQ as ineffective and potentially harmful.\textsuperscript{51} HCQ with or without AZM did not decrease ventilation risk significantly. The HCQ group, but not the HCQ + AZM group, even had a higher risk for death. However, patients with severe disease were potentially more likely to start HCQ treatment. The dosage, duration, and consistency of regimen across the study centers were not reported. A New York hospital reported QTc prolongation associated with HCQ + AZM (N = 84).\textsuperscript{52} QTc increased from a baseline of 435 ± 24 ms to a maximal value of 463 ± 32 ms (P < 0.001) on day 3.6 ± 1.6 of therapy. No TdP events were observed.

Currently, the National Institutes of Health recommends neither for nor against CQ /HCQ as a treatment for COVID-19.\textsuperscript{53} However, the organization does not support the HCQ + AZM combination due to potential toxicity. With the ongoing research effort for COVID-19, results from well-designed RCTs are expected to evaluate the efficacy and safety of CQ and HCQ against SARS-CoV-2.

### Pharmacokinetics

Both CQ and HCQ follow linear kinetics.\textsuperscript{6} Peak level of both drugs following oral administration is achieved in 2-4 hours with an average of 89% (CQ) and 74% (HCQ) dose absorption.\textsuperscript{6,54} It is worth noting that a 30%-100% variation in CQ and HCQ absorption has been observed among individuals.\textsuperscript{6,55}

Half-lives of CQ and HCQ are between 40 and 50 days.\textsuperscript{6,54} HCQ can be detected in urine 3 months after a single oral dose of 200 mg.\textsuperscript{54} The long t1/2 is attributed to extensive tissue binding. The volumes of distribution for both drugs are reportedly thousands of liters.\textsuperscript{15,55}

CQ and HCQ are metabolized partially through the liver with the primary metabolites being desethylchloroquine and desethylhydroxychloroquine, respectively.\textsuperscript{54,55} CQ and HCQ are subjects of metabolic enzymes CYP2C8, CYP3A4/5, and CYP2D6.\textsuperscript{15,56} About 16%-30% HCQ is excreted via kidney unchanged and does not appear correlated with creatinine clearance.\textsuperscript{54} While short-term use does not require renal dosing, prolonged use may warrant dose reduction in renal impairment.\textsuperscript{54,55,57}

### Adverse Effects and Toxicities

In 1947, the first human toxicity studies noted electrocardiographic (EKG) changes and visual changes in 20 subjects taking CQ 0.3 g daily for 77 days.\textsuperscript{58} Researchers have found that CQ/HCQ can impede conduction in sodium and potassium channels, leading to ST-segment depression, T wave inversion, and QT interval prolongation. QT interval prolongation is a particular concern when given concomitantly with AZM in COVID-19, as this combination increases the risk of torsades de pointes (TdP) and sudden cardiac death.\textsuperscript{59-62} CQ/HCQ can also cause a complete atrioventricular block.\textsuperscript{62} These cardiotoxicities have been seen after chronic daily use as well as in acute overdoses of HCQ (≥8 g) or more commonly in CQ (≥2.25 g).\textsuperscript{61,63,64}

Since the 1960s, research on visual changes revealed that chronic use of CQ and HCQ could irreversibly impair retinal function. A recent study demonstrated that CQ and HCQ (10 μM) inhibit organic anion transporting polypeptide 1A2 (OATp1A2), leading to the accumulation of all-trans-retinol in

### Table 1. Clinical Applications of Chloroquine and Hydroxychloroquine.

| Pharmacological properties | Clinical applications (Established and investigative) |
|---------------------------|-----------------------------------------------------|
| Antimalarial               | Malaria                                             |
| Anti-inflammatory          | Antiphospholipid syndrome                           |
|                           | Inflammatory bowel disease                          |
|                           | Rheumatoid arthritis                                |
|                           | Systemic lupus erythematosus                        |
|                           | Sjogren’s Syndrome                                  |
| Antitumor                  | Blood cancers (leukemia, myeloma, lymphoma)         |
|                           | Brain cancers                                       |
|                           | Breast cancer                                       |
|                           | Gastrointestinal cancers                            |
|                           | Genitourinary cancers                               |
|                           | Lung cancer                                          |
|                           | Skin and bone cancers                               |
| Antiviral                  | Coronaviruses                                        |
|                           | Ebola                                               |
|                           | Hepatitis                                           |
|                           | Human immunodeficiency virus                        |
|                           | Zika                                                |

(Will added more if applicable)
human retinal pigment epithelium cells. The American Academy of Ophthalmology recommends an annual screen after 5 years of treatment of daily CQ 2.3 mg/kg or HCQ 5 mg/kg (dosed at actual body weight). Other major adverse effects of CQ/HCQ include dermatologic reactions, gastrointestinal discomfort, and a decrease in blood cell counts.

Conclusion and Outlook
CQ and HCQ have been around for decades. Ever since they were first synthesized, CQ and HCQ have remained as valuable therapeutic agents worldwide. Medical indications for CQ and HCQ have expanded from malaria to inflammatory diseases, now to cancers and emerging viral infections (Table 1). The research effort to explore their efficacy and new mechanism of action in various diseases has never ceased. Along with that, kinetics studies are ongoing to balance toxicity and effectiveness. Despite their long history in modern medicine, CQ and HCQ continue to be meaningful objects of study.

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