Chloroquine and Hydroxychloroquine for the Prevention and Treatment of COVID-19; a Fiction, Hope or Hype? An Updated Review

Sultan Saghir[1], Naif AlGabri[2,3], Mahmoud Alagawany[4], Youssef Attia[5,6], Salem Alyileili[7], Shaaban S. Elnesr[8], Manal Shafi[9], Omar Al-shargi[10], Nader Al-balagi[11], Abdullah Alwajeeh[12], Omar Alsalahi[13], Amlan K. Patra[14], Asmaa Khafaga[15], Ahmed Negida[16,17], Ahmed Noreldin[18], Wesam Al-Amarat[19], Amer A. Almaiman[20], Khaled A. El-Tarabily[21,22*] and Mohamed Abd El-Hack[4]*

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[1]Department of Medical Analysis, Princess Aisha Bint Al-Hussein College of Nursing and Medical Sciences, Al-Hussein Bin Talal University, 71111, Ma'an, Jordan, [2]Pathology Department, Faculty of Veterinary Medicine, Thamar University, Dhamar, Yemen, [3]Laboratory of Regional Djibouti Livestock Quarantine, Abu Yasar International Est. 1999, Djibouti, [4]Department of Poultry, Faculty of Agriculture, Zagazig University, 44511, Zagazig, Egypt, [5]Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University, 21589, Jeddah, Kingdom of Saudi Arabia, [6]Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour, Egypt, [7]Department of Integrative Agriculture, College of Food and Agriculture, United Arab Emirates University, 15551, Al-Ain, United Arab Emirates, [8]Department of Poultry Production, Faculty of Agriculture, Fayoum University, 63514, Fayoum, Egypt, [9]Department of Biological Sciences, Zoology, King Abdulaziz University, 21589, Jeddah, Kingdom of Saudi Arabia, [10]Department of Pharmacology, College of Pharmacy, Riyadh Elm University, Riyadh, Kingdom of Saudi Arabia, [11]Ministry of Health, Riyadh, Kingdom of Saudi Arabia, [12]Anti-DopingLab, Doha, Qatar, [13]Department of Medical Laboratories, Faculty of Medicine and Health Sciences, Hodeidah University, Al Hodaidah, Yemen, [14]Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences,

Belgachia, Kolkata, India, [15]*Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, 22758, Edfina, Egypt,* [16]*School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK,* [17]*Zagazig University Hospitals, Zagazig University, Zagazig, Egypt,* [18]*Histology and Cytology Department, Faculty of Veterinary Medicine, Damanhour University, 22511, Damanhour, Egypt,* [19]*Department of Medical Support, Al-Karak University College, Al-Balqa' Applied University, Jordan,* [20]*Department of Applied Medical Sciences, Community College of Uniazah,Qassim University, Buraydah 51431, Kingdom of Saudi Arabia,* [21]*Department of Biology, College of Science, United Arab Emirates University, 15551, Al-Ain, United Arab Emirates,* [22]*College of Science, Health, Engineering and Education, Murdoch University, Murdoch, 6150, W.A., Australia.*

[*]**Correspondence:**

Youssef A. Attia (yaattia@kau.edu.sa), and Khaled A. El-Tarabily (ktarabily@uaeu.ac.ae)

SIMPLE SUMMARY

Understanding the role of chloroquine and hydroxychloroquine in COIVD-19 treatment is crucial for effective disease treatment. Knowledge of the prophylactic nature and positive effects of these treatments will allow optimization of treatment time and cost. Since the outbreak of COVID-19 in December 2019, screening for drugs and vaccines for COVID-19 treatment gained increasing interest. Drug interventions are a strategy for disease prevention and control. This will help patients, physicians, health care agents, and pharmaceutical industries to develop an effective plan for sustainable human health care during the COVID-19 crisis. Hence, we aimed to explore chloroquine and hydroxychloroquine's fundamental role as a regimen for COVID-19 patients' with or without azithromycin, illustrating the structure, mechanism of action, and side effects and drug interactions, data of experimental studies, and data of clinical trials.

ABSTRACT

In December 2019, the novel coronavirus disease pandemic (COVID-19) that began in China had infected more than 56 million individuals worldwide and accounted for more than 1.344.000 fatalities. With the dawn of this novel coronavirus (SARS-CoV-2), there was a requirement to select potential therapies that might effectively kill the virus, accelerate the recovery, or decrease the case fatality rate. Besides the currently available antiviral medications for HIV and HCV, the chloroquine/hydroxychloroquine (CQ/HCQ) regimen with or without azithromycin has been repurposed in China and was recommended by the National Health Commission, China in mid-February 2020. By this time, the selection of this regimen was based on its efficacy against the previous SARS-CoV-1 virus and its potential to inhibit viral replication of the SARS-CoV-2 *in vitro*. There was a shortage of robust clinical proof about the effectiveness of this regimen against the novel SARS-CoV-2. Therefore, extensive research effort has been made by several researchers worldwide to investigate whether this regimen is safe and effective for the management of COVID-19. This review article provides a comprehensive overview of the CQ/HCQ regimen. It summarizes the evaluating data from *in vitro* studies and clinical studies either for the protection or the treatment against SARS-CoV-2. There is a sharp difference of opinion about the role of CQ/ HCQ regimen in treatment of COVID-19. The literature data are controversial and contradictory due to the diverse study design, population selection, dosage, regimen, and outcome measures. Current evidence from the two largest randomized-controlled trials (recovery and solidarity) suggests that the HCQ regimen does not decrease COVID-19 patients' mortality. However, conflicting data were published from observational studies showing that the drug might be sufficient. Therefore, more investigations are needed to emphasize these findings.

Keywords: Chloroquine, COVID-19, drug safety, hydroxychloroquine, SARS-CoV-2, treatments

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is a disease pandemic caused by a new strain of coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Formerly, this disease was referred to as '2019 novel coronavirus' or '2019-nCoV.' The virus name (SARS-CoV-2) was chosen because the virus is genetically related to the coronavirus responsible for the SARS outbreak of 2003. While related, the two viruses are different (WHO, 2020a). The spread of SARS-CoV-2 began in Wuhan, China, by the end of December 2019. As of November 20, 2020, the COVID-19 pandemic has swept the world and infected more than 56 million individuals worldwide and accounted for more than 1.344.000 fatalities (WHO, 2020b).

The initial case fatality rate of this virus was estimated to be 2% but ranged in some countries to 4 to 9%. After adjustment for asymptomatic cases, this virus's actual fatality rate was estimated to be around 1%. The major challenge of COVID-19 is the rapid transmission of the virus and the substantial proportion of asymptomatic individuals who account for 40-50% of transmission (Liu et al., 2020).

Extensive efforts are being made to fight this virus, including both pharmacological and non-pharmacological interventions. In the search for potential pharmacologic agents that might be useful to protect against the virus and/or treat COVID-19 patients, clinicians have repositioned chloroquine (CQ) and hydroxychloroquine (HCQ) as a treatment regimen. The rationale for selecting this regimen in the early months of the pandemic was the following: (1) This regimen has been previously utilized for the cure against SARS-CoV-1 with documented success, and (2) recent *in vitro* experiments in China showed that these agents could inhibit viral replication *in vitro* (Liu et al., 2020).

Since then, this regimen has divided the world with one extreme trolling it as 'game changer in medicine' while other touting it as 'useless and dangerous'. Therefore, in the present article, we provide a comprehensive review of the use of CQ/HCQ regimen with or without azithromycin, illustrating the structure, mechanism of action, side effects and drug interactions, and experimental studies data, and data of clinical trials.

Structure of the SARS-CoV-2 Virus

Coronaviruses are spherical with an average diameter of 80-120 nm. They possess a number of club-shaped (17-20 nm) glycoproteins spikes projecting from the surface of the viral envelope (Chan et al., 2020). The virus particle contains five major structural proteins, which are glycoprotein spikes (S), an envelope protein (E), matrix protein (M) and nucleocapsid (N) protein. The glycoprotein spikes mediate virus's attachment to different host cell receptors, depending upon the receptor-binding domain (RBD). On attachment to the host cell receptor, the glycoprotein spikes S protein cleavages into two subunits, namely, N-terminal S1 and C-terminal S2 subunit regions by the host proteases enzyme. S1 subunit contains a signal peptide and a RBD. Meanwhile S2 subunit contains conserved fusion peptide (FP), heptad repeat (HR) peptides, transmembrane domain (TM) and a cytoplasmic domain (Chan et al., 2020).

The S1 subunit of SARS-CoV-2 showed 70% identity to Beta coronavirus's S1 subunits (SARS-CoV-1) isolated from human and bats. Human angiotensin-converting enzyme 2 (hACE2), acts as the key receptor to infect the human cells (Chen, 2020). The S2 subunit plays an important role in mediating the virus fusion and entry into the host cell, in which heptad repeat 1 and 2 (HR1, HR2) can interact with six helical bundles, thereby bringing the viral and cellular membrane in close proximity for fusion (Chen, 2020).

The ACE2-binding affinity of RBD in S1 subunit of SARS-CoV-2 is 10 to 20-fold higher, which might contribute to the higher infectivity and transmissibility of SARS-CoV-2

compared to SARS-CoV-1. The M glycoprotein is pre-glycosylated M polypeptides with a size range of 25-30 kDa (221-262 amino acids) and gives shape to the virus envelope. Envelope protein (E) is a small polypeptide with a size range of 8.4-12 kDa (76-109 amino acids) and is the integral membrane protein (Chan et al., 2020; Chen, 2020).

Chemical Compositions and Sources

CQ and HCQ have similar chemical structures and cellular mechanisms of action (Liu et al., 2020). CQ is administered as a phosphate salt, whereas HCQ is administered as a sulphate. Both drugs are absorbed in the upper intestinal tract (Schrezenmeier & Dörner, 2020). The CQ is produced by systematic modification of quinine, which is a plant alkaloid and quinoline containing compound (Bawa et al., 2020). Hans Andersag and co-workers discovered CQ in 1934 at the Bayer laboratory and named it "Resochin". It became available in clinical practice in 1947 and quickly became the drug of choice for the treatment of malaria (Bawa et al., 2020).

CQ, 7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline is made by reacting 4-diethylamino-1-methylbutylamine with 4,7-dichloroquinoline at 180 °C (Drake et al., 1964). Each of the two components involved in CQ synthesis can be prepared in several ways (Figure 1). In 1946, HCQ sulphate was synthesized as a derivative of CQ by incorporating a hydroxyl group into CQ, and they both share comparable mechanisms of action as weak bases and immuno-modulators and chemical structures (Liu et al., 2020).

It was proved that HCQ is (~40%) toxic compared with CQ in animals (McChesney, 1983). More interestingly, HCQ, compared with CQ, is vastly available to cure auto-immune diseases like rheumatoid arthritis and systemic lupus erythematosus (Colson et al., 2020).

Mechanism of Action

Both CQ and HCQ are weak bases that increase the pH of acidic intracellular organelles like lysosomes/endosomes that require low pH for maturation and function (Mauthe et al., 2018).

CQ showed elevation of pH in lysosomes from nearly 4.5 to 6.5 at 100 μM. However, the effect of HCQ on pH values of lysosomes/endosomes is not known due to the lack of studies in this regard (Liu et al., 2020).

Moreover, CQ was found to cause changes in the glycosylation of angiotensin-converting enzyme 2 (ACE2) spike protein and receptor, that ultimately inhibits the entry step, and the post-entry phase of SARS-CoV-2 (Chen et al., 2020a). HCQ in the time-of-addition experiment showed its ability to exert the same mechanism (Figure 2).

In addition to the previously known mechanism, a novel mechanism of action for CQ and HCQ on COVID-19 was discovered in 2020 by Fantini et al. (2020) as it is known that SARS-CoV-2 starts its replication by attaching to the spike (S) viral protein of respiratory cells. The S protein utilizes sialic acids and ACE-2 receptor connected to host cell surface gangliosides for entry. The study showed that CQ (or its more active derivative, HCQ) has a high affinity for binding to gangliosides and sialic acids (Fantini et al., 2020).

The study also distinguished a novel ganglioside-binding domain (111–158) at the tip of the N-terminal domain of the SARS-CoV-2 S protein. It is expected that this domain can ease attachment with the ACE-2 receptor and enhance contact of the virus to lipid rafts (Fantini et al., 2020).

Side Effects of the CQ/HCQ Treatment

High doses of CQ were found to cause severe side effects, but it was reported that CQ in a prescribed dose exerts relatively few adverse effects (Goel & Gerriets, 2020). Ocular adverse effects such as long and subtle symptoms of reduced visual acuity, diplopia, retinal toxicity, and bilateral loss of vision were found to be the most severe side effects caused by high doses of CQ (Praga et al., 2020). A high dosage of CQ also causes critical psychiatric issues such as hallucinations, paranoia, and suicidal ideations (Lysack et al., 1998). Injecting CQ intramuscularly has shown to cause potentially life-threatening hypotension (White, 1998).

Other adverse effects include pruritus, photosensitivity, seizures, paranoia, hallucinations, and retinopathy characterized by the inability to focus on near and far objects (Juurlink, 2020) (Figure 3). HCQ has a more solubility and less toxic metabolites compared with CQ. Hence it has fewer adverse effects and is relatively safer (Sahraei et al., 2020). For these reasons, HCQ is often preferred over CQ where possible (Juurlink, 2020).

Cautions and Contraindications

Patients receiving CQ or/and HCQ must be monitored for their haematological parameters (RBC, WBC, and platelet counts), blood glucose (hypoglycemic risk of HCQ), serum electrolytes, renal as well as hepatic functions (Singh et al., 2020). Electrocardiography (ECG) is essential before starting therapy with these medications and the concomitant use of these drugs with other drugs known to extend the corrected QT (QTc) interval of the heart (like antihistamines, anti-depressants, anti-arrhythmic, anti-psychotics, moxifloxacin, teneligliptin, and ondansetron) should be averted (FDA, 2020). The addendum of HCQ to azithromycin, as reported by Gautret et al. (2020) in the French trial, may elevate QTc extension (Chorin et al., 2020a). If QTc is 450–500 msec, it is recommended to do daily ECG. CQ and HCQ must not be utilized simultaneously with ritonavir/lopinavir and remdisivir for expected QTc extension. Additionally, hypoglycaemia should be observed in diabetes patients, particularly with concomitant usage of CQ/HCQ and ritonavir/lopinavir. Pharmacovigilance on the mental and visual disorder is also carefully wanted (Figure 4).

Despite case reports of reversible heart failure and CQ-induced cardiomyopathy in the literature, large meta-analysis and numerous investigations carried out in patients having rheumatoid arthritis confirmed a lowered cardiovascular hazard with both drugs; none the less, a baseline ECG must be completed in patients with certain cardiovascular disease (Cortegiani et al., 2020). Every clinician utilizing these drugs should realize contraindications to both compounds; porphyria, pre-existing maculopathy, retinopathy, glucose-6-phosphate

dehydrogenase deficiency, epilepsy, recent myocardial infarction, hypersensitivity to these agents, and QTc>500 msec (Singh et al., 2020). There is no evidence that CQ and HCQ are contraindicated in lactating and pregnant women (Dashraath et al., 2020).

It is worth noticing that CQ and HCQ interact with various drugs; many lead to QT prolongation and might lead to serious cardiac events and death. As mentioned earlier, this includes patients who take the CQ/HCQ regimen with azithromycin. Such patients require close cardiac monitoring as long as they are on the CQ/HCQ regimen. Besides, CQ/HCQ might decrease blood glucose; therefore, these drugs can be used with caution in patients with diabetes mellitus. A recent study showed that using these drugs during the pandemic contributed to hypoglycaemic events (Shah et al., 2020).

A summary of the common drug and disease interactions of CQ and HCQ are shown in Table 1.

Experiment Studies

The continuous and rapid spread of the COVID-19 pandemic has led to extensive ongoing efforts worldwide to develop effective and safe therapy. CQ and HCQ in COVID-19 are among the drugs being tested, which were reported on February 4, 2020, to suppress SARS-CoV-2 *in vitro*.

There is considerable *in vitro* evidence that CQ and HCQ are efficient in preventing SARS-CoV-2 vigour. Liu et al. (2020) detected that both drugs have a 50% cytotoxic concentration (CC50). However, the 50% maximum efficient concentration was lower for CQ than HCQ (EC50 – the dose at which viral RNA elevation is suppressed by 50%) regardless of the multiplicity of infection (MOI – the ratio of virions to host cells) (Liu et al., 2020).

Wang et al. (2020) found that CQ has *in vitro* antiviral vigour with an EC50 of 1.13 μM and CC50 >100 μM at an MOI of 0.05 and shown that the eclecticism for SARS-CoV-2 is

high compared with that for host cells. The study also showed that CQ at a concentration of 0.36 mg/L decreased viral load by 50% *in vitro* using Vero E6 cells (Wang et al., 2020).

Yao et al. (2020) also proved the activity of CQ versus SARS-CoV-2 and detected that CQ was less potent than HCQ *in vitro* versus SARS-CoV-2 (EC50 of 5.47 μM and 0.72 μM, respectively, MOI = 0.01). Based on PBPK models results, oral HCQ sulfate with a supplying dose of 400 mg twice a day then 200 mg twice a day as a maintenance dose for four days is advised for SARS-CoV-2 infection, and it is three times more potent than CQ phosphate when given 500 mg twice per day for five days in advance (Yao et al., 2020).

Clinical Trials on CQ/HCQ Regimen for the Protection against SARS-CoV-2 Infection

Although preclinical evidence suggests that CQ and HCQ can inhibit viral replication and might prevent COVID-19, the current evidence does not support their prophylaxis efficacy against SARS-CoV-2 infection (Wang et al., 2020).

Expert opinions advised using the CQ/HCQ regimen for prophylaxis against SARS-CoV-2 infection, particularly between healthcare labors who are at higher hazard of infection (Cohen, 2020; Tilangi et al., 2020). However, this opinion was refuted by data from a well-designed randomized controlled trial on 821 participants. Participants were allocated to be administrated with either HCQ or placebo within four days after exposure. The happening of novel symptoms compatible with COVID-19 did not vary markedly among the two groups (11.8% versus 14.3%; P=0.35) (Poulware et al., 2020).

Clinical Experiments on CQ/HCQ Regimen for the Therapy of COVID-19

Recent literature has suggested that CQ/HCQ drugs could be used as antiviral drugs to cure COVID-19 infections (Cortegiani et al., 2020). In addition, Iyer et al. (2020) stipulated that the CQ can block the quinone reductase-2, a fundamental agent needed for the sialic acid biosynthesis that SARS-CoV-2 utilizes it as the receptor moieties. A recent small clinical study by Gautret et al. (2020) reported that positive SARS-CoV-2 in nasopharyngeal

secretions significantly decreased on day six after inclusion in HCQ-treated COVID-19 patients against patients who received supportive care only (Gautret et al., 2020).

The CQ elevates pH in host cell lysosomes and passively affects virus–receptor linking and intervenes with the glycosylation of SARS-CoV-2 receptors. Additionally, it showed a hopeful antiviral influence versus SARS-CoV-2 *in vitro* and limited the course of the disease and enhanced COVID-19-pneumonia patients (Gao et al., 2009).

The first evidence of CQ effectiveness in COVID-19 came from China in February 2020 by the Chinese government (Gao et al., 2020). These data reported that CQ phosphate was given to over 100 patients in China and reduced the duration of illness and significantly improved pneumonia infection and lung imaging. There were no adverse events reported. It seems that combining data from various in-progress trials using a variety of study designs released such findings.

A study by Gautret et al. (2020) in France on March 17, 2020, considered as the first clinical trial, was conducted as an open-label non-randomized controlled experiment. The trial included patients who suffered from SARS-CoV-2 among which 22 of the 36 patients included in the study had symptoms in the upper respiratory tract, eight had symptoms in the lower respiratory tract, while six patients were asymptomatic. The experimental group (22 patients) was treated with HCQ 200 mg three times per day for ten days, whereas the control group treated with ordinary care. Azithromycin was also prescribed for six patients of the treatment group to prevent bacterial superinfection. In this trial, SARS-CoV-2 carriage at day 6 was the primary outcome which was examined by testing nasopharyngeal swabs utilizing PCR of SARS-CoV-2 RNA (Gautret et al., 2020).

The experiment's outcomes revealed that the experimental group was markedly tested negative for the virus than patients in the control group (70% vs. 12.5% virologically cured, $p<0.001$) on day 6. Furthermore, the results of HCQ and azithromycin combination were

astonishing as all patients treated with this combination were negative on day 6. The study proves the efficiency of HCQ and the possible synergistic influence of its combination with azithromycin needs further declaration, as suggested by Gautret et al. (2020).

Despite this trial's favourable outcomes, severe limitations have made its results questionable (Ullah et al., 2020). First, there was recruitment for an additional six patients but were excluded, and no intention-to-treat analysis was performed due to many reasons that have led to the failure of following-up these patients (Ranganathan et al., 2016; Ullah et al., 2020). Secondly, the researchers added that the sample size was not enough to achieve 85% power, which required recruiting 48 patients for the required power to be achieved. The overstatement of influence sizes and false-positive outcomes can be expected from the underpowered trial with a sample size of 36 patients (Dumas-Mallet et al., 2017). On the sixth day, the researchers reported that a patient showed negative for the virus but revealed positive on the eighth day, which raised a concern about a trial lacking for long-term and medium follow-up data since the primary outcome is viral PCR status at day 6. This incidence indicates that long-term data of CQ/HCQ effectiveness in the therapy of COVID-19 is necessary. Finally, the trial's allocation bias cannot be denied where there was no randomization for patients to the control and treatment group (Dumas-Mallet et al., 2017).

Another pilot study published on March 25, 2020, by Chen et al. (2020b) who evaluated the safety and efficacy of HCQ in the management of patients with COVID-19. A sum of 30 patients diagnosed with COVID-19 was recruited and randomly allocated (1:1) into the treatment and control groups. The test group treated with oral CQ sulfate (400 mg one time a day for five days) based on conventional treatment, while the control group received traditional treatment. The principal outcome was the negative change rate of COVID-19 nucleic acid in respiratory pharyngeal swab on the seventh day. On day 7, the test group's throat swabs showed negative COVID-19 nucleic acid in 13 patients (86.7%), with one case

progressed to severe during the treatment (Chen et al., 2020b). In comparison to the treatment group, 14 (93.3%) subjects in the control group (P>0.05) also tested negative. The average period between virus nucleic acid negative maintenance and patients' hospitalization in the test and control groups was 4 (1-9) days and 2 (1-4) days, respectively (U=83.5, P>0.05) (Chen et al., 2020b). In terms of safety, abnormal liver function and transient diarrhea in the experimental group and the control subjects were noticed in 4 (26.7%) and 3 (20%) cases, respectively (P>0.05). The small sample size in this study has made a general conclusion that the prediction of typical COVID-19 patients is perfect (Chen et al., 2020b).

Following that, an extensive argument was raised against Gautret et al. (2020) study by Kim et al. (2020). It was reported that there was a rush in judgment of the study due to the pressing requirement for efficient therapy for SARS–CoV-2. The clinical trial's limitations were discussed, such as using an invalidated replacement endpoint, deficiency of blinding or randomization, and including the small sample size. Another study highlighted methodological flaws that were considered to impact the validity of the findings (Dahly et al., 2020).

Despite the limitations in the first clinical trial, its promising results ended up advising the usage of CQ/HCQ in the management of COVID-19 officially by guidelines. The National Health Commission published the recommendation of treatment COVID-19 by CQ, China, published in mid-February 2020, indicating that 500 mg CQ phosphate (equivalent to 300 mg CQ) twice per day for ten days is recommended for patients with COVID-19 (Dong et al., 2020). On March 17, 2020, other recommendations published by the L. Spallanzani National Institute for Infectious Disease in Italy, in which the combination of CQ (500 mg CQ per day) or HCQ (HCQ per day, 200-500 mg/day) with a different antiviral drug is indicated for COVID-19 (Nicastri et al., 2020).

A pharmacokinetic study in France aimed to optimize HCQ dosing in the intensive care unit (ICU) of COVID-19 patients was carried out by Perinel et al. (2020). The study recruited 13 patients in ICU who were treated by HCQ at a dose of 200 mg twice per day. The mean age of patients was 68 years, 31% with moderate or severe renal failure, and 46% were obese. The study demonstrated that the dosing regimen of 200 mg thrice a day is inappropriate to reach a supposed target blood level of 1 – 2 mg/L in this population. According to data from patients with rheumatoid arthritis and the 161 blood levels registered, the proposed dosing regimen delivers a dose of 800 mg once per day on the first day, then 200 mg twice per day for seven days (Perinel et al., 2020).

The efficacy of combining azithromycin and HCQ was also evaluated by an uncontrolled non-comparative observational study carried out by Gautret et al. (2020) in 80 patients diagnosed with a restively mild infection of COVID-19. Six days were set as the minimum follow-up period. There was a clinically marked amelioration in all patients, except for one patient aged 86 years who died, and another patient (74-year-old) was still in the ICU. The viral load of nasopharyngeal samples rapidly decreased. Of the samples, 83% of patients were tested negative on the seventh day, while on the eight's day, 93% were negative (Gautret et al., 2020). On day 5 of the treatment, respiratory samples' viral cultures were found negative in 97.5% of patients. Therefore, patients were quickly got out of the infectious disease unit with five days as an average length of stay. Although the number of patients was just 80 and the severity of the illness was mild, the study reflected an excellent picture of the combination of azithromycin and HCQ (Gautret et al., 2020).

Regarding the optimal dose of HCQ in COVID-19 patients, Garcia-Cremades et al. (2020) tested the safe and effective dosage of HCQ for COVID−19 treatment. It was predicted that doses of over 400 mg twice a day of HCQ for ≥five days reduced viral loads quickly, shortening the treatment course, decreasing the number of patients with detectable

SARS-CoV-2 infection. In contrast, increasing the dose of HCQ to over 600 mg twice a day has more probability of prolonging QTc intervals (Garcia-Cremades et al., 2020). In recent study from Belgium, Catteau et al. (2020) have shown that the low dose HCQ monotherapy has reduced mortality rate compared with the non-HCQ treated patients (Catteau et al., 2020).

A study from South Korea (Lee et al., 2020) investigated the effectiveness of post-exposure prophylaxis after a significant exposure of COVID-19 in a long-term care hospital using HCQ (400 mg orally daily till the end of 14 days of quarantine) in 211 persons containing 22 healthcare workers and 189 patients, with negative PCR checks for COVID-19 (Lee et al., 2020). After completing the post-exposure prophylaxis period by 184 patients and 21 care-workers without any severe effects, all PCR tests were negative at the ending of the 14 days of quarantine (Lee et al., 2020). The shortage of control groups in the study and having other 29 hospital staff who tested negative after the 14 days of quarantine although they did not receive post-exposure prophylaxis (Although being classified low-risk exposure) are considered essential limitations in the study (Lee et al., 2020).

In a study highlighted COVID-19 and immunomodulation in inflammatory bowel diseases (IBD), Neurath (2020) mentioned that there is a possibility for drug-drug interactions between HCQ or IBD therapies. The risk of interaction is potentially increased by combination of medication with HCQ and infliximab/adalimumab for nerve harm (Neurath, 2020).

However, there is no evidence to discontinue IBD-specific medications in COVID-19 patients cured with such drugs. The favourable effect of HCQ and azithromycin combination on the clinical results and viral loads of patients infected with COVID-19 has led to implementing the regimen by clinicians worldwide. On the other hand, both drugs have been independently revealed to influence the electrical system of the heart, causing QT-interval

elongation, drug-induced *torsades de pointes*, and drug-stimulated sudden cardiac death (Chen et al., 2006).

In this context, an American study (Chorin et al., 2020b) examined the QT-interval in 84 patients with COVID-19 cured with a combination of HCQ (400 mg daily on day one, then 200 mg daily from day 2 to 5) and azithromycin (500 mg per day for five days). After 4.3 ± 1.7 days as an average time for exposure to HCQ/azithromycin, ECG was followed up. It was found that the QTc markedly extended. In a group of nine (11%) of those patients, there was a severe prolongation of the QTc to >500 ms, which is a marker of a high danger of sudden cardiac death caused by malignant arrhythmia (Chorin et al., 2020b). Out of the group of nine patients, five patients had a normal QTc. It was suggested that regular evaluation for QTc must be implemented by patients with COVID-19 who are cured with a combination of HCQ/ azithromycin combination, especially those who have comorbidities or/and with other QT-prolonging medications (Chorin et al., 2020b).

A randomized clinical experiment by Borba et al. (2020) from Brazil compared the effect of high doses (600 mg twice per day for ten days) against small doses (450 mg twice a day on day one and OD for four days) of CQ diphosphate as adjunctive therapy for 81 adult patients treated with SARS-CoV-2 infection (Borba et al., 2020). Forty patients received low doses, while 41 received high doses. In the small dose group, 15.0% (6 out of 40) of patients died on day 13 days compared with 39% of the high dose group (16 of the 41 patients). Regarding safety, 4 of 36 patients (11.1%) receiving low-dose experienced prolongation of QTc interval compared with 7 of 37 (18.9%) patients receiving the high-dose (Borba et al., 2020). Besides, ventricular tachycardia was developed in 2 patients (2.7%) in the high-dose group. As a result of these findings, the trial was stopped. It was inferred that the high dosage of CQ must not be advised for adversely ill patients with COVID-19 (Borba et al., 2020).

Patients with systemic lupus erythematosus (SLE) were a population of interest for Mathian et al. (2020). SARS-CoV-2 represents a source of concern for the management of patients with SLE. In patients with SLE, the use of immunosuppressive drugs, the intrinsic perturbations of the immune response, and the potential presence of organ damage associated with their disease make those patients at higher risk of severe infections. Currently, and as a part of SLE treatment, HCQ is a standard long-term drug for SLE (Savarino et al., 2003).

HCQ also has antiviral activity in COVID-19, and its therapeutic or even prophylactic activity for COVID-19 was proved by preliminary clinical trials. Mathian et al. (2020) examined the clinical observations of COVID-19 in a series of 17 patients with SLE receiving long-term treatment of HCQ (median of 7.5 years) and with obesity and chronic kidney disease as comorbidities. Although this study gave an initial clinical view of the infection course in patients with SLE cured with HCQ, it did not conclude the severity and incidence rate of COVID-19 in SLE. Moreover, it was also shown that HCQ does not protect against COVID-19, at least its negative practice, in patients with SLE (Mathian et al., 2020).

On the other hand, strong evidence from a well-designed randomized controlled trial (RCT) does not advocate the usage of CQ/HCQ regimens in COVID-19 patients. Data from the UK's recovery trial, the world's largest COVID-19 clinical trial to date, showed that HCQ did not reduce the 28-day mortality rate among COVID-19 patients compared to the standard of care (Horby & Landray, 2020). While these outcomes were questioned by several experts owing to the relatively higher loading dose.

On the first day of the study (2400 mg in 24 hours), similar findings were reached by the WHO's solidarity trial in several countries worldwide. On June 5, 2020, the WHO announced that based on an interim analysis of the trial data, HCQ did not reduce the mortality compared to the standard of care (WHO, 2020c).

The characteristics of the *in vitro* studies on SARS-CoV-2 and clinical trials studying the efficacy of CQ and HCQ in COVID-19 patients are illustrated in Table 2.

Past Experiences, Current Situations, and Future Directions

Based on the review of the existing literature, the CQ/HCQ regimen gained worldwide attention. It showed a promise in the preclinical experiments and some clinical studies during the early months of the pandemic. Nonetheless, the usage of the CQ/HCQ regimen in treating COVID-19 has been challenged by the recent data from well-designed RCTs. The CQ and HCQ are widely used for the first-line of treatment against the malarial parasite in most endemic Asia and African countries (Mushtaque & Shahjahan, 2015). Besides malaria treatment, CQ is utilized in rheumatoid arthritis, systemic and discoid lupus erythematosus, sarcoidosis, scleroderma, pemphigus porphyria cutanea tarda (Mushtaque & Shahjahan, 2015). Despite drugs' adverse effects on humans, such as cardiac, retinal, and neuromuscular toxicities, their benefits outweigh the toxicity effects (Taylor & White, 2004; Plantone & Koudriavtseva, 2018). The CQ and HCQ have also been tested to treat various diseases such as human immunodeficiency diseases, Q fever, whipple disease, and fungal infection (Plantone & Koudriavtseva, 2018; Bonam et al., 2020). These drugs have several other beneficial properties, including anti-inflammatory, immuno-modulating, anti-infective, anti-thrombotic, and anti-tumoral properties (Plantone & Koudriavtseva, 2018).

Due to these multifaceted effects of CQ and HCQ, including antiviral properties, these drugs have been extensively investigated against the SARS-COV-2 virus and COVID-19 patients, and the outcomes widely varied. Indeed, few *in vitro* investigations have revealed antiviral influences against SARS-COV-2 (Liu et al., 2020; Wang et al., 2020; Yao et al., 2020). The results are preliminary based on the small clinical trials and usually cofounding with pre-existing comorbidities, age, and severity of disease (Mathian et al., 2020).

The prophylaxis use of CQ and HCQ has not been proven to show real effects due to insufficient data in a standard trial. In most cases, there is a lack of robust experimental designs and randomized control trials with long-term supervision of the patients. Many times, its toxicity, particularly cardiac toxicities, outweighed its benefits, unlike the treatment of malarial infection. A recent meta-analysis of 12 studies showed no evidence of clinical benefit from CQ/HCQ administration in COVID-19 patients (Ullah et al., 2020). Other limitations of this regimen were (1) the potential interaction with azithromycin and several other medications leading to QT prolongation and possible cardiovascular side effects and (2) the hypoglycemia if not adequately monitored in diabetic patients. While close monitoring might optimize is regimen's safety, the safety profile does not make it suitable for a pandemic situation. With several cases overwhelming the healthcare systems, it becomes unpractical to screen all patients for the potential interactions in the clinical setting.

Future directions in the CQ/HCQ drugs might include improved drug delivery either by inhalation (Tai et al., 2020) or trans catheter delivery through the bronchial artery (Zaitoun et al., 2020). Ivermectin is a medication applied to control different types of parasite infestations, although recently repurposed and getting attention owing to its low cost and wide availability for COVID-19 treatment (Caly et al., 2020; Chowdhury et al., 2020; Gorial et al., 2020).

Ivermectin and other avermectins (insecticides most frequently used in home-use ant baits) are macrocyclic lactones derived from the actinobacterium *Streptomyces avermitilis*. Ivermectin kills by interfering with the nervous system and muscle function, particularly by enhancing inhibitory neurotransmission (Chowdhury et al., 2020). However, reliable data from well-designed studies are needed to guide clinical practice.

Therefore, there is a need for longitudinal studies using a large number of populations in different countries using standard randomized controlled studies to validate therapeutic

benefits and apparent efficacy against different clades of SARS-CoV-2 virus and COVID-19 and their relative safe uses under specified conditions. The risk-benefits ratios from these studies should be appropriately analyzed before it could be recommended to treat COVID-19 patients. The randomized and controlled WHO Solidarity (Pan et al., 2020) trial did not find an effectiveness of HCQ in reducing mortality rate (risk ratio of 1.19; P = 0.23) among the hospitalized COVID-19 patients. Based on lack of benefits of using HCQ, WHO (WHO, 2020c) and National Institute of Health had stopped trial for hospitalized COVID patients (Horbey et al., 2020a). A recent randomized controlled trial by Horbey et al. (2020a) in the UK comprising of 4716 COVID-19 patients showed that administration of HCQ had no benefits in decreasing death rate (rate ratio of 1.09; P = 0.15). Moreover, a recent meta-analysis based on 28 randomized trial containing 10.012 COVID-19 patients treated with HCQ, 307 patients with CQ and 63 patients with both CQ and HCQ in which WHO Solidarity (Pan et al., 2020) and RECOVERY (Horbey et al., 2020b) included that HCQ treatment was associated with increased (risk ratio of 1.11; P = 0.02) mortality rate, whereas CQ did not show (risk ratio of 1.77; P = 0.21) any benefit in reducing mortality rate (Axfors et al., 2020). Finally, according to new data from two large RCTs (Recovery and Solidarity), the FDA revoked the CQ/HCQ regimen's emergency usage authorization in COVID-19 patients. The drugs are currently used for clinical trial purposes only (FDA, 2020).

CONCLUSION AND RECOMMENDATIONS

Based on the available evidence of CQ and HCQ for treatment of SARS-CoV-2, both have received emergency usage authorization from the FDA for COVID-19 on March 28, 2020. However, the two largest RCTs data to date showed no clinical advantage of HCQ treatment in COVID-19 patients. As a result, the FDA revoked the emergency use authorization of this regimen. In terms of prophylaxis, one RCT showed no evidence of post-exposure prevention from COVID-19. Despite the fact, CQ/HCQ has been desperately used for treatment of

COVID-19 in clinical practices. The benefits of using HCQ as prophylaxis or in patients with less severe COVID-19 patients managed in the community seem not conclusive. Nonetheless, based on the reports of large randomized controlled trials of RECOVERY (Horbey et al., 2020a) and WHO SOLIDARITY (Pan et al., 2020) and meta-analysis studies, it is not currently advisable to use this regimen outside the purpose of approved clinical trials for hospitalized COVID-19 patients.

DATA AVAILABILITY STATEMENT

This review article is based on the published available literature.

ETHICS STATEMENT

The review was carried according to International Guidelines for research involving animals (Directive 2010/63/EU).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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795 **Table 1. The commonest drug interactions and disease interactions of the chloroquine**
796 **(CQ) and Hydroxychloroquine (HCQ) regimen.**

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	CQ	HCQ
Drug interactions	▪ Hydroxyzine ▪ Azithromycin ▪ Ciprofloxacin ▪ Duloxetine ▪ HCQ ▪ Levetiracetam ▪ Pregabalin ▪ Mefloquine ▪ Primaquine ▪ Albuterol	▪ Amitriptyline ▪ Calcium/Vitamin D ▪ Duloxetine ▪ Leflunomide ▪ Albuterol ▪ Tramadol
Disease interactions	▪ Oculotoxicity ▪ Porphyria ▪ Arrhythmias ▪ Bone marrow suppression ▪ Ototoxicity ▪ Seizures ▪ Glucose-6-PD deficiency ▪ Hepatotoxicity ▪ Myasthenia gravis ▪ Psoriasis	▪ Oculotoxicity ▪ Porphyria ▪ Arrhythmias ▪ Bone marrow suppression ▪ Ototoxicity ▪ Seizures ▪ Glucose-6-PD deficiency ▪ Hepatotoxicity ▪ Myasthenia gravis ▪ Psoriasis ▪ Diabetes ▪ Heart disease ▪ Renal impairment

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809 **Table 2. Characteristics of the *in vitro* investigations on SARS-CoV-2 and clinical trials**
810 **studying the efficacy of chloroquine and hydroxychloroquine in COVID-19 patients.**

Reference and country	Population (n patients)	Intervention and comparison groups	Primary outcomes
(Yao et al., 2020); China	*in vitro* study with SARS-CoV-2-infected Vero cells	Infected Vero cells were treated with CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for 24 or 48 h.	• CQ and HCQ decreased viral replication in a concentration-dependent manner. • EC_{50} values for CQ were 23.90 and 5.47 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.14 and 0.72 μM at 24 and 48 h, respectively.
(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
(Gautret et al., 2020); France	Age >12 years and positive for SARS-CoV-2. Patients with HCQ or CQ allergy were excluded or had another recognized contraindication to cure with the drug. Pregnant and breastfeeding patients were excluded.	Oral HCQ 200 mg TD × ten days (n=20). Symptomatic treatment and AZT (n = 6; 500 mg/d on day 1 then 250 mg/d for next 4 days) with HCQ. Patients (n=16) who rejected the cure or had relegation criteria, served as controls.	• Control patients were younger than HCQ-treated patients (37.3 years vs 51.2 years). • At sixth day post-inclusion, 70% of HCQ-cured patients were negative compared with 12.5% in the control group (p= 0.001). • At day six post-inclusion, 100% of patients treated with combination of HCQ and AZT were negative compared with 57.1% in patients cured with HCQ only, and 12.5% in

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			the control group ($p<0.001$).
(Chen et al., 2020b); China	Confirmed COVID-19 patients. Thirty patients were randomly grouped into treatment and control groups.	Oral HCQ sulfate 400 mg OD × 5 days (n=15). No HCQ was provided to patients (n=15).	• On day 7, the number of negative samples did not differ (13 (86.7%) cases in the HCQ group versus 14 (93.3%) cases in the control group; $P>0.05$) • The period from hospitalization to negative result of virus nucleic acid did not differ (4±1.9 days in HCQ versus 2±1.4 days in the control group; $P>0.05$). • The time for body temperature normalization was comparable (1±0.2 day I HCQ group versus 1±0.3 days in the control group).

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(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
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			• Radiological progress was noted on CT images in 7 cases (46.7%) of the control group and 5 cases (33.3%) of the HCQ group, and all patients revealed amelioration in follow-up examinations. • Three cases (20%) of the control group and four cases (26.7%) of the HCQ group had abnormal liver function and transient diarrhoea (P>0.05).
(Lee et al., 2020); South Korea	COVID-19 exposed individuals (211 containing 22 careworkers and 189	COVID-19 exposed individuals were administered HCQ at 400 mg OD x 14 days	• At the ending of two weeks of quarantine, all follow-up PCR tests were negative. • A sum of 32 individuals (15.6%)

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	patients) with negative PCR tests for COVID-19 in a long-term care hospital in Korea. Four patients and one coworker were not finally completed.	during the quarantine. No control groups.	mentioned one or more symptoms through post-exposure prophylaxis. • The most common symptoms were skin rash (4.3%), loose stool or diarrhoea (9%), bradycardia (0.95%), and gastrointestinal upset (0.95%). Post-exposure prophylaxis was stopped in 5 patients (2.7%) because of the requirement for fasting (1), bradycardia (2), and gastrointestinal upset (2).
(van den Proek et al., 2020); Netherlands	Patients (n = 95) were aged 18 years or older and suspected of having COVID-19	CQ was a loading dose of 600 mg followed by 300 mg BD (starting 12 h after the loading	• CQ treatment in patients with COVID-19 markedly extended the QTc interval by 34–35 ms; 23% of patients had a QTc interval exceeding 500 ms.

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	disease.	dose), for the next four days	Statistically marked influences were detected on QRS interval (mean difference 6 ms), PR interval (mean difference 8 ms), and heart rate (mean difference –10 bpm).
(Chorin et al., 2020a); Netherlands	A retrospective investigation of 251 patients having COVID-19.	HCQ was orally administrated at 400 mg BD for one day (loading dose) then 200 mg BD for four days. AZT was orally administrated for five days at a dose of 500 mg OD.	• The QTc interval extended from a baseline of 439 ± 29 ms to a maximum value of 473 ± 36 ms ($P < .001$), which happen on day 4.1 ± 2 of treatment. • Extreme novel QTc interval extension to >500 ms revealed in 23% of patients. • One patient showed polymorphic ventricular tachycardia.

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(Geleris et al.., 2020); USA	A retrospective investigation of 1376 patients having COVID-19.	HCQ (n = 811) was provided at 600 mg BD on day 1, followed by 400 mg/d for 4 next days. Control group patients were less adversely ill at baseline than those with HCQ-treated patients (n = 565; the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, 223 vs 360).	• HCQ use was not accompanied with a markedly lower or higher hazard of death or intubation (hazard ratio, 1.04; 95% CI, 0.82 to 1.32).

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(Magagnoli et al., 2020); USA	A retrospective investigation of 368 patients diagnosed with COVID-19.	HCQ (n = 97) alone and HCQ + AZT (n = 113) in combination. In the control group (n = 158), no HCQ was provided.	• The hazard of death from any reason was elevated in the HCQ group (adjusted hazard ratio, 2.61; 95% CI, 1.10 to 6.17; P=0.03). • The risk of death was similar in the HCQ+AZ group (adjusted hazard ratio, 1.14; 95% CI, 0.56 to 2.32; P=0.72). • The hazard of ventilation wascomparable in the HCQ group (adjusted hazard ratio, 1.43; 95% CI, 0.53 to 3.79; P=0.48) and the HC+AZ group (adjusted hazard ratio, 0.43; 95% CI, 0.16 to 1.12; P=0.09).
(Mahevas et al.,	A retrospective	HCQ (n = 84) 600 mg/	• 20.2% of patients in the HCQ group

Reference and country	Population (n patients)	Intervention and comparison groups	Primary outcomes
(Yao et al., 2020); China	*in vitro* study with SARS-CoV-2-infected Vero cells	Infected Vero cells were treated with CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for 24 or 48 h.	• CQ and HCQ decreased viral replication in a concentration-dependent manner. • EC_{50} values for CQ were 23.90 and 5.47 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.14 and 0.72 μM at 24 and 48 h, respectively.
(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
2020); France	investigation of 181 patients having COVID-19 and requiring oxygen ≥ 2 L/min.	d for 7 day In control (n = 97), no HCQ was provided.	were died within seven days or moved to the ICU vs 22.1% in the no-HCQ group (16 vs 21 events, the relative hazard of 0.91, 95% CI 0.47-1.80) in the HCQ group. • The death of 2.8% of the patients was within seven days vs 4.6% in the no-HCQ group (three vs four events, the relative risk of 0.61, 95% CI 0.13-2.89). • 27.4% in the HCQ group versus 24.1% in control group patients developed acute respiratory distress syndrome within seven days (24 vs 23 events, relative risk of 1.14, 95% CI

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(Yao et al., 2020); China	*in vitro* study with SARS-CoV-2-infected Vero cells	Infected Vero cells were treated with CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for 24 or 48 h.	• CQ and HCQ decreased viral replication in a concentration-dependent manner. • EC_{50} values for CQ were 23.90 and 5.47 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.14 and 0.72 μM at 24 and 48 h, respectively.
(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
			0.65-2.00). • 8 patients receiving HCQ (9.5%) revealed electrocardiogram modificationsrequesting HCQ stop.
(Rosenberg et al., 2020); USA	A retrospective investigation of 181 patients having COVID-19.	HCQ at 200–600 mg OD/BD (n = 271) alone; HCQ + AZT (n = 735) in combination; AZT 200–500 mg once/ OD/BD (n = 211), and no drug (n =221)	• The death of patients treating with AZT alone, 21/211 (10.0% (95% CI, 5.9%-14.0%)), HCQ + AZT was 189/735 (25.7% (95% CI, 22.3%-28.9%)), HCQ alone, 54/271 (19.9% (95% CI, 15.2%-24.7%)), and neither drug, 28/221 (12.7% (95% CI, 8.3%-17.1%)). • Co marked variations in mortality for patients receiving HCQ + AZT (hazard ratio of 1.35 (95% CI, 0.76-2.40)),

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(Yao et al., 2020); China	*in vitro* study with SARS-CoV-2-infected Vero cells	Infected Vero cells were treated with CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for 24 or 48 h.	• CQ and HCQ decreased viral replication in a concentration-dependent manner. • EC_{50} values for CQ were 23.90 and 5.47 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.14 and 0.72 μM at 24 and 48 h, respectively.
(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
			HCQ alone (hazard ratio of 1.08 (95% CI, 0.63-1.85)), or AZT alone (hazard ratio of 0.56 (95% CI, 0.26-1.21)) in comparison with patients administrating neither drug. • Cardiac arrest was markedly higher in patients receiving HCQ + AZT (adjusted OR, 2.13 (95% CI, 1.12-4.05)), but not HCQ alone (adjusted OR, 1.91 (95% CI, 0.96-3.81)) or AZT alone (adjusted OR, 0.64 (95% CI, 0.27-1.56)) compared with patients receiving neither drug.
(Yu et al., 2020); China	A retrospective investigation of 181	HCQ 400 mg/d (200 mg BD) for 7–10 days	• Mortalities reduced in HCQ group (18.8% (9/48) versus 45.8% (238/520)

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(Yao et al., 2020); China	*in vitro* study with SARS-CoV-2-infected Vero cells	Infected Vero cells were treated with CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for 24 or 48 h.	• CQ and HCQ decreased viral replication in a concentration-dependent manner. • EC_{50} values for CQ were 23.90 and 5.47 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.14 and 0.72 μM at 24 and 48 h, respectively.
(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
	patients having COVID-19 and treated with HCQ.	(n = 48). In the control group (n = 520), no HCQ was provided.	in control group (p<0.001)). • The time of hospitalization before patient death was 15 (10-21) days for the HCQ group versus 8 (4 - 14) days for control groups (p<0.05). • The level of inflammatory cytokine IL-6 markedly decreased from 22.2 (8.3-118.9) pg/mL to 5.2 (3.0-23.4) pg/ml (p<0.05) in the HCQ group, but there is no alteration in the control group.
Recovery trial (Horby et al., 2020a); UK	An ongoing randomized controlled trial of more than 11,000 COVID-19	HCQ(200 mg tablet containing 155 mg base equivalent) received a loading	• 28-day mortality was 26.8% and 25% in the HCQ and standard of care groups. • HCQ treatment was markedly

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(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
	patients to date	dose of four tablets (800 mg) at zero and six hours, then two tablets (400 mg) starting at twelve hours after the initial dose and then every twelve hours for the next nine days or until discharge.	accompanied with an elevated length of hospital stay and elevated hazard of developing to death.
Solidarity trial, (WHO, 2020c)	An ongoing randomized controlled trial of more than 5,000 COVID-19 patients to	HCQ Standard of care	• Not Available; Details were not published.

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(Yao et al., 2020); China	*in vitro* study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher *in vitro* antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
(Liu et al., 2020); China	*in vitro* study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
(Wang et al., 2020); China	*in vitro* study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50}> 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat, (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
	date		
(Skipper et al., 2020); US and Canada	An internet-based randomized controlled trial in non-hospitalized patients in the US and Canada	HCQ(800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 more days) Placebo	• Symptom severity did not significantly differ over 14 days (−0.27 points (95% CI, −0.61 to 0.07 points); P=0.117). • At 14 days, 24% of participants receiving HCQ had ongoing symptoms compared with 30% receiving placebo (P=0.21). • Medication adverse effects occurred in 43% of HCQ group compared to 22% in the placebo group (P < 0.001).

HCQ, hydroxychloroquine; CQ, chloroquine; OD, one a day; BD, twice a day; TD, thrice a day; CI, confidence interval; EC50, Half maximal effective concentration; CC50, 50% cytotoxic concentration. SI, selectivity index.

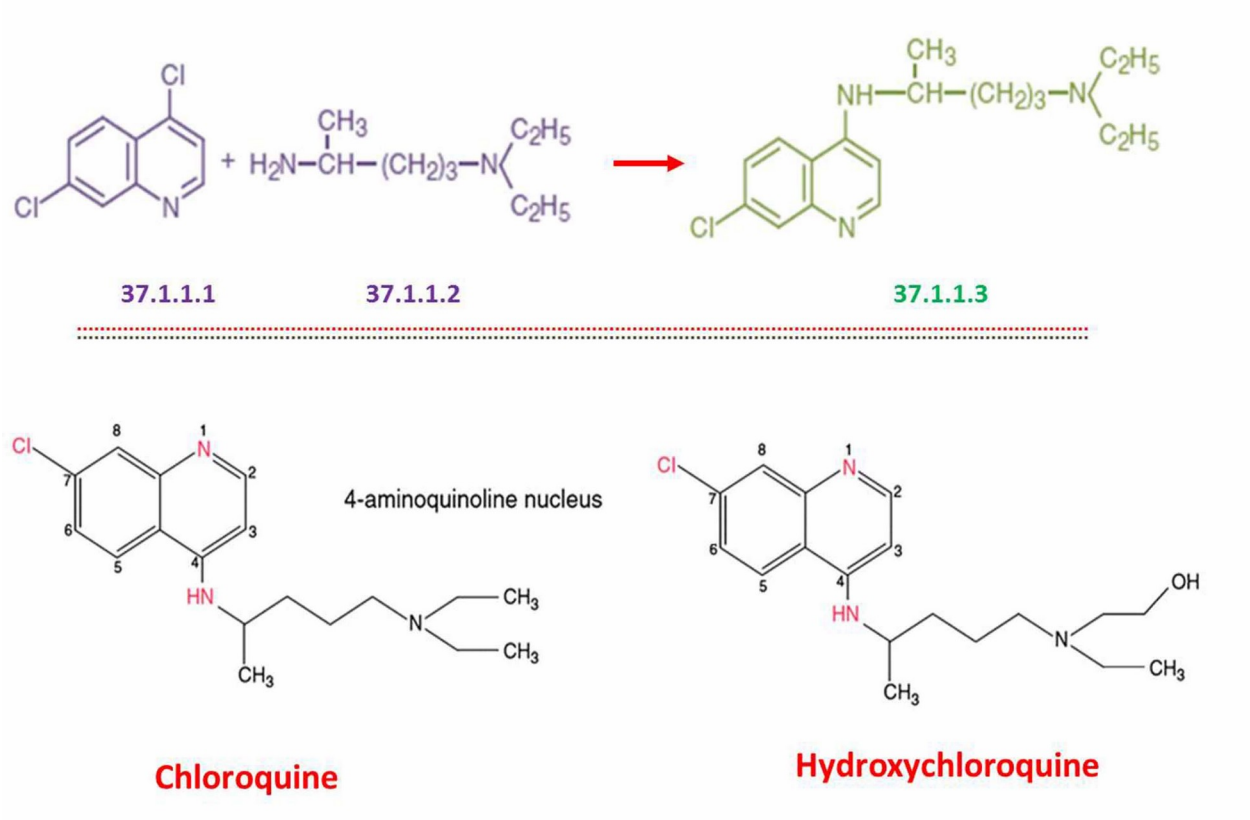


Figure 1. Chemical composition of chloroquine and hydroxychloroquine.

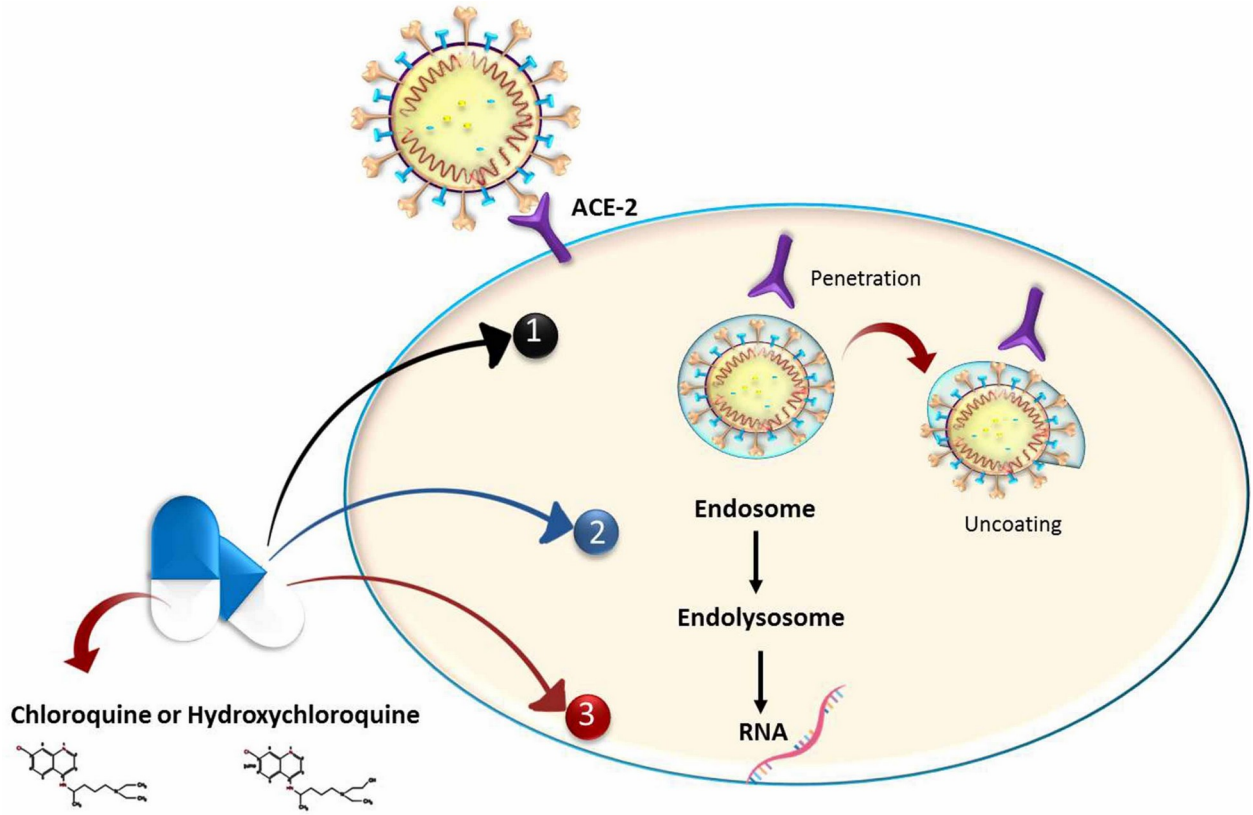
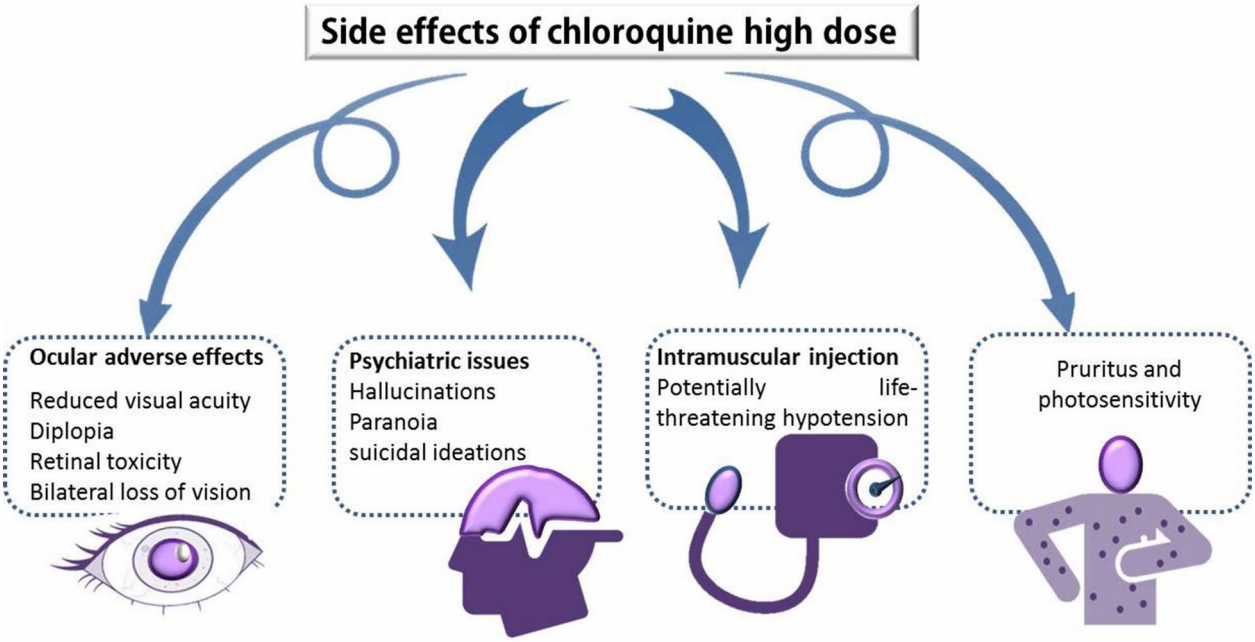


Figure 2. The possible mode of action of chloroquine and hydroxychloroquine versus SARS-CoV-2 infection: (1) interference with the terminal glycosylation of cellular receptor ACE-2 leads to obstructing virus-receptor attachment; (2) increasing the pH of acidic cellular organelles lead to prevention of endocytosis with adverse influences on post-translational modification of recently synthesized viral RNA and virion transport; (3) blocking of viral protein synthesisand virion assembly.



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837 **Figure 3.** The possible side effects of chloroquine and hydroxychloroquine.

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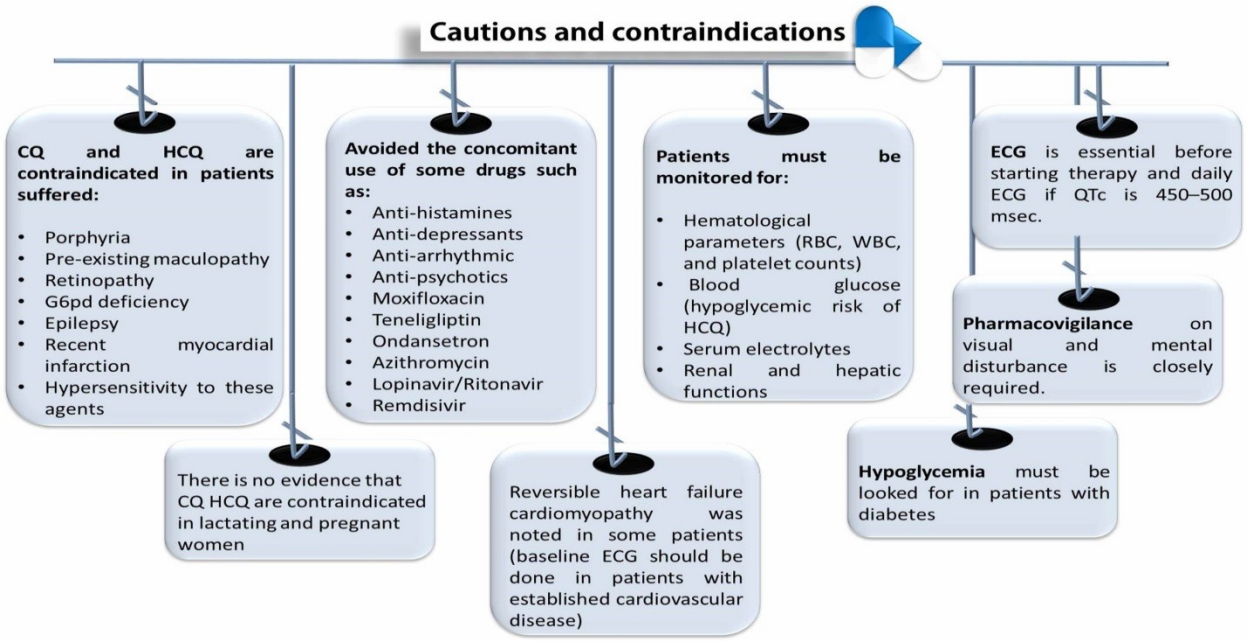

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848 **Figure 4.** Cautions and contraindications during treatment with chloroquine and
849 hydroxychloroquine.

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