A pharmacological perspective of Chloroquine in SARS-CoV-2 infection

An old drug for the fight against the new coronavirus?

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Key words: SARS-CoV-2; COVID-19; Chloroquine; Hydroxychloroquine; Antiviral

Abstract

The pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is having serious consequences on health and the economy worldwide. All evidence-based treatment strategies need to be considered to combat this new virus. Drugs need to be considered on scientific grounds of efficacy, safety and cost. Chloroquine (CQ) and hydroxychloroquine (HCQ) are old drugs used in the treatment of malaria; in addition, their antiviral properties have been previously studied, including in coronaviruses, where evidence of efficacy has been found. The safety of CQ and HCQ has been studied for over 50 years. In the current race against time triggered by the SARS-CoV-2 pandemic, the search for new antivirals is very important. However, consideration should be given to old drugs with known anti-coronavirus activity, such as CQ and HCQ; these could be integrated into current treatment strategies while novel treatments are awaited, also in light of the fact that they display an anticoagulant effect that facilitates the activity of low MW heparin, aimed at preventing ARDS-associated thrombotic events.

The safety of CQ and HCQ has been studied for over 50 years, however, recently published data raise concerns for cardiac toxicity of CQ/HCQ in patients with COVID-19. The review that we here provide also reexamines the real information provided by some of the published alarming reports although concluding that cardiac toxicity should in any case be stringently monitored with patients with CQ/HCQ**.**

Keywords: SARS-CoV-2; COVID-19; Chloroquine; Hydroxychloroquine; Antiviral

Introduction

On December 31st, 2019, twenty-seven cases of pneumonia of unknown etiology were reported in the city of Wuhan, Hubei province in China which quickly spread to various countries [1,2]. On February 7th, 2020, the causative agent was identified and named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which the World Health Organization (WHO) had named as COVID-19. When we started to conduct this review study, on March 11th, 2020, WHO declared the outbreak of the new SARS-CoV-2 as a pandemic [3]; on that date, 129,775 cases of infection had been reported in 114 countries, with 4,751 deaths and 68,672 people recovered. People affected by COVID-19 infection can have a wide range of respiratory infection symptoms, including fever, shortness of breath and cough, from asymptomatic or very mild to severe pneumonia. Mortality until March 3rd was calculated at 3.4%. On vaccine development, as of February 23rd, 2020, there were 15 phase I clinical trials. On the other hand, 23 clinical trials had been registered with different antivirals, monoclonal antibodies,

Methylprednisolone, Teicoplanin and among all these, two with Chloroquine. In the Chinese Clinical Trial Registry (http://www.chictr.org.cn) six studies of Chloroquine (QC) and Hydroxychloroquine (HCQ) for the treatment of SARS-CoV-2 were reported to be in progress[4–6].

CQ and HCQ are antimalarials that belong to the group of aminoquinolines. HCQ differs from CQ by the presence of a hydroxyl group at the end of the side chain. HCQ is available for oral administration in the sulfate form. CQ and HCQ are old antimalarial drugs, but in the current context, their potential antiviral properties are of interest[7]. The present review aims at describing the pharmacological basis and potential therapeutic utility of CQ and HCQ in SARS-CoV-2 infection.

History

CQ is an antimalarial drug synthesized in Germany in 1934, emerging as a substitute for natural quinine, which is extracted from the bark of the quinine tree (*Cinchona officinalis*). The healing properties of the bark of the quinine tree were discovered by the ancient Incas; for that reason, it is the national tree of Peru and appears in the national coat of arms. Its name comes from Chinchon, the countess wife to the Spanish viceroy, who in 1638 was cured of malaria with the bark of this tree and began to spread its use throughout the world. CQ is a cheap, well-known medicine that has been used for more than 50 years. Although it had been widely used in the treatment of malaria, the appearance of CQ-resistant plasmodium has decreased its use in this disease[8,9]. In 1946, HCQ was synthesized and was shown to be much less toxic than CQ in animals[10].

SARS-CoV-2

Coronaviruses (CoV) infect birds and mammals. Human coronaviruses (HCoV) generally cause respiratory and intestinal infections of low severity, with two notable exceptions that occurred in 2002 and 2012[1]. In 2002, a new virus emerged in Guangdong, southern China, which caused severe acute respiratory syndrome (SARS)[11]. This virus was called the SARS-CoV coronavirus and it caused 8,000 human infections and 774 deaths in 37 countries during 2002–03[12]. In 2012, the Middle East Respiratory Syndrome (MERS) coronavirus emerged, which was first detected in Saudi Arabia[13], producing 2494 laboratory-confirmed cases of infection and 858 deaths, 38 of which were in South Korea[14,15]. The SARS-CoV-2 virus that appeared in December 2019 is the seventh human coronavirus found to cause respiratory infection and it belongs to the genus

Betacoronavirus originating from bats. SARS-CoV-2 has approximately 79% sequence similarity with SARS-CoV and 50% with MERS-CoV[2]. SARS-CoV-2 is postulated to use the Angiotensin Converting Enzyme 2 (ACE2) receptor to infect the human cell, based on its similarity to SARS-CoV in its receptor binding domain structure [2,16]. Wang et al. have reported that the infection mechanism based on the use of the human ACE2 cell receptor is common in SARS-CoV, MERS-CoV and SARS-CoV-2; however, there may be a difference with SARS CoV-2, in that the latter has the ability to increase the expression of ACE2 in the host cell, which facilitates its infection and spread[17].

SARS-CoV-2 structure

The structure of the SARS-CoV-2 virion is comprised by a spike glycoprotein (S), a hemagglutinin-esterase dimer (HE), a membrane glycoprotein (M), an envelope protein (E), the nucleocapsid protein (N) and the RNA genome[2]. The S glycoprotein is highly glycosylated and uses an N-terminal signal sequence to enter the endoplasmic reticulum (ER) and bind to receptors of the human host cell. The S glycoprotein determines the tissue tropism of the virus, that is, the SARS-CoV-2 affinity towards the host cell. SARS-CoV-2 binds to the ACE2 receptor expressed in pneumocytes[17,18]. The binding to the ACE2 receptor triggers conformational changes in the S glycoprotein, allowing its cleavage by the transmembrane protease TMPRSS2 of S glycoprotein and the release of S fragments in the cell supernatant, which inhibit virus neutralization by antibodies[19]. Coronaviruses are so named because the S glycoprotein that surrounds the virus forms large bumps giving the impression of a crown (from the Latin “corona”, in turn derived from the Greek “Korone”)[20,21]. In most coronaviruses, S is cleaved by a furin-like protease from the host cell into two separate polypeptides, S1 and S2. The nucleocapsid (N) protein binds to RNA in vitro, is highly phosphorylated and has the function of binding the viral genome to the replicase-transcriptase complex (RTC) and subsequently packaging the genome encapsulated in viral particles. The envelope glycoprotein (E) is probably a transmembrane protein, with functions of acting as an ion channel, facilitating the assembly and release of the virus. Membrane protein (M) is present as a dimer in the virion and can have two different conformations to allow promote membrane curvature and joining the nucleocapsid. Finally, the hemagglutinin esterase (HE) dimeric glycoprotein binds to sialic acids in surface glycoproteins[16].

Pharmacodynamics

Studies have shown that CQ/HCQ may have antiviral action through the following mechanisms (Fig 1):

Prevention of virus entry into the cell. Many viruses invade the cell using the endocytic pathway[22,23]. CQ alters the pH of endosomes and therefore may have an inhibitory effect on viral infections such as those causing Borna disease[24], avian leukosis[25], Zika[26], influenza[27], Japanese encephalitis [28]and dengue[29,30].

Altered virus replication. Viruses use the host cell machinery to produce their progeny. Some enveloped viruses additionally require posttranslational modifications of the envelope glycoproteins for the formation of new viruses; this occurs within the endoplasm and vesicles of the trans-Golgi network (TGN). This complex process requires enzymes such as proteases and glycosyltransferases, which in some cases require a medium with a low pH. By raising the pH of endosomes, CQ / HCQ may cause dysfunction of several enzymes among which are glycosyltransferases. This mechanism may explain possible effects of CQ / HCQ inhibiting budding of Mayaro virus particles[31], inducing the accumulation of non-infectious herpes simplex virus 1 particles in the TGN[32]and inhibiting the replication of viruses of the family Flaviviridae by affecting the proteolytic process of conversion of prM to M protein of flavivirus[33]. *In-vitro* and *in-vivo* studies have suggested that CQ alters the glycosylation pattern of the HIV-1 gp120 envelope and inhibits replication of HIV in CD4+ T cells, producing non-infectious retrovirus particles[34–37].

Inhibition of autophagy. Animal studies have suggested that CQ can inhibit autophagy in the lungs of mice with H5N1 avian influenza and reduce alveolar epithelial damage [38]. In mice studies, CQ can prevent vertical transmission of the Zika virus by maternal-fetal pathway [39].

Immune-modulating activity. The CQ/HCQ-induced pH elevation in cellular organelles may have the effect of inhibiting the production of various cytokines, chemokines, or mediators, an excessive activity of which is pathophysiologically related to the severity of viral infections. By reducing the excessive production of these mediators of inflammation, CQ/HCQ may have an immunomodulatory effect. CQ/HCQ is currently used in the treatment of autoimmune-based diseases such as rheumatoid arthritis and systemic lupus erythematosus. The

main mechanism of this immunomodulatory action is partly mediated by the reduction of tumor necrosis factor (TNF) at the level of monocyte-macrophages[40–42].

Anticoagulant activity

An anticoagulant activity of aminoquinoline drugs has been reported since the 1960's [43]. CQ was reported to inhibit the alternative pathway of complement as well as to abrogate the clotting of plasma by calcium chloride and thrombin [44]. However, these activities were reported in vitro at CQ concentrations superior to those likely to be obtained in human plasma at therapeutically acceptable dosages. In 2019, Miranda et al. reported an inhibitory effect of CQ on coagulation in vivo through impairment of the extrinsic pathway, i.e. by impairing tissue factor (TF) release from the endothelium[45]. In this regard, the anticoagulant activity of HCQ can be seen as a byproduct of its anti-inflammatory activity. This is in line with anticoagulant effects of the drug reported in individuals with lupus erythematosus[46]. The anticoagulant activity of HCQ mainly targeting the extrinsic pathway, may thus be complementary to that of low-molecular weight heparin (LMWH) , which targets, among other mechanisms, the intrinsic pathway by inhibiting the activation of factor X by factor IXa [47]. As inhibition of the TF/factor VIIa pathway by HCQ also has repercussions on activation of factor X [48] the HCQ/LMWH combination may exert a synergistic inhibition of coagulation converging in factor X and impeding in thrombus formation during COVID-19. This drug combination has become part of the standard of care in Italy[49].

Specific Anti-SARS-CoV-2 potential mechanisms of action

As outlined above, CQ/HCQ may have anti-SARS-CoV-2 action through three general mechanisms: prevent viral entry, impair replication, and a pleiotropic action on the human immune system through immuno-modulating activity. More specifically, SARS-CoV-2 requires to interact with and bind to human cellular receptors for entry into the host cell, and in this process the ACE2 receptor and the transmembrane protease play key roles. CQ/HCQ may also affect the latter.

Possible CQ / HCQ mechanism of action at the ACE2 receptor level. Previous studies in SARS-CoV discovered a binding affinity between the ACE2 receptor and the S glycoprotein [50]. The mechanism of action of CQ against SARS-CoV may be the induction of surface expression of sub-glycosylated ACE2, as the alteration of terminal

glycosylation of ACE2 decreases the binding affinity between the human ACE2 receptor and the SARS-CoV and the S glycoprotein, thus preventing the entry of virus to the cell[51]. Xu *et al.* found that the receptor-binding domain of SARS-CoV-2 S glycoprotein has a strong interaction with human ACE2 molecules, despite its sequence diversity with its homologue encoded by SARS-CoV [52]. In fact, the affinity of ACE2 for SARS-CoV-2 is much higher than for SARS-CoV, which explains why the former seems to be more easily transmitted [47]. Wang *et al.* have reported that SARS-CoV-2 can increase ACE2 expression in lung tissue, so that the same virus may potentiate and accelerate its replication and dissemination processes, in a fashion similar to that observed for SARS-CoV and MERS-CoV [17]. CQ/HCQ attenuates the effects of this overexpression of ACE2, so that the replication and dissemination of SARS-CoV-2 is reduced[51–53].

Possible CQ / HCQ mechanism of action at the transmembrane protein level. CQ / HCQ inhibit quinone reductase 2[54], a protein sharing structural homology with UDP N-acetylglucosamine 2-epimerase, an important enzyme in sialic acid biosynthesis[37]. The catalytic site of the latter enzyme is consistent with binding of a chloroquine molecule, as shown by molecular docking[37]. Through this mechanism, CQ / HCQ may decrease the biosynthesis of sialic acid, which is required for the surface to which SARS-CoV-2 binds, before entering the host cell[53].

Possible inhibition of coronavirus papain-like protease (PLpro). A provocative study, though not yet peer reviewed, revealed, by *in-silico* molecular docking, an unexpected potential target for chloroquine, i.e. PLpro, which is one of the two viral cysteine proteases involved in post translational cleavage of SARS-CoV-2 proteins[55]. If these *in-silico* predictions are confirmed, this would be noteworthy, as the association of CQ/HCQ with lopinavir, a drug combination originally proposed by one of us against SARS[41]and recommended by several national guidelines for COVID-19 treatment (see below) might target the two main viral proteases simultaneously. The other cysteine protease of SARS-CoVs, *i.e.* the 3-chymotrypsin-like protease (3CL-pro), is the putative target for lopinavir, originally developed as an anti-HIV drug[56].

Immunomodulatory activity. In the immunopathogenesis of severe cases of SARS, a phenomenon that worsens the damage caused by viruses is called "inflammatory storm"[57]. Severe systemic and pulmonary inflammation in SARS patients has been postulated to be the result of dysregulation in the levels of cytokines

such as TNF-α, IP-10, IL-6, and IL-8[58,59]. A similar phenomenon called “cytokine storm” has been observed in patients with SARS-CoV-2, because they display high levels of IL-1B, IFN-γ, IP-10 and MCP1, which probably lead to activated T-helper-1 cell responses. Patients with severe SARS-CoV-2 infection requiring admission to the Intensive Care Unit (ICU) had higher concentrations of G-CSF, IP-10, MCP1, MIP1A, MIP1 and TNF-α than those who did not require admission to the ICU, suggesting that the "cytokine storm" was associated with the severity of the disease[60]. In line with the self-limiting nature of the disease in a significant proportion of patients, SARS-CoV-2 infection may also initiate increased secretion of T-helper-2 cytokines (e.g. IL-4 and IL-10) that suppress inflammation, a phenomenon which differs from SARS-CoV infection. In the pathophysiology of this “cytokine storm” associated with SARS-CoV-2, the ACE2 receptor seems to play an important role. The hypothesis that ACE2 is a gene sensitive to virus infection especially by SARS-CoV-2 has been proposed; the inducibility of ACE2 by inflammatory cytokines also implies that the "cytokine storm" caused by 2019-nCoV not only damages the host tissues but can also accelerate the spread of the virus[60,61]. Therefore, induction by CQ/HCQ of ACE2 subglycosylation could hypothetically have immunomodulating effects related or not to the aforementioned inhibition by CQ of cytokine production, chemokines and other mediators of inflammation.

Pharmacokinetics

CQ and HCQ have similar pharmacokinetics, with rapid gastrointestinal absorption and renal elimination. From many years of experience in malaria, two main differences between the two drugs are known: CQ is toxic at high doses (therefore it is typically used at higher doses for a short time or low doses over a long period), whilst HCQ can be used in high doses for long periods with very good tolerance[53]. After oral administration, CQ / HCQ are widely and slowly distributed throughout the body, and this is due to extensive sequestration in tissues, particularly in liver, spleen, kidney, lung, melanin-containing tissues and, to a lesser extent, brain and spinal cord[62]. This large apparent volume of distribution confers to CQ/HCQ a relatively short plasma half life. CQ/HCQ accumulates in many cell types. Cell permeation by CQ/HCQ can be deduced by studies conducted in human erythrocytes and *Plasmodium falciparum* cells [63–65]. CQ and HCQ are weak bases, the main cell permeant is the unprotonated form of CQ which represents a minority of the extracellular CQ pool. Due to the Henderson-Hasselbach equation, however, part of the remaining CQ portion dissociates to maintain equilibrium at the physiological pH, thus allowing the drug to gradually enter the cells. As passage through the plasma membrane is due to diffusion and not to active transportation, the process does not become saturated, and the

initial intracellular accumulation of the drug is dose-dependent. This pharmacokinetic property allows administering loading doses in order to reach the desired intracellular concentrations more quickly. Once inside the cells, CQ/HCQ is protonated at a rate inversely proportional to the pH, again, according to the Henderson-Hasselbach law[36].

Within the intracellular compartment, the drug is actively transported to the acidic intracellular organelles where a large amount of the drug becomes entrapped due to protonation associated with the low pH. CQ and HCQ enter the endosome, Golgi vesicles and lysosomes, where the pH is low, and in this medium most of the CQ and HCQ molecules are positively charged[66]. In whole blood the drug is approx. 4-5 fold more concentrated than in plasma due to this intracellular accumulation [67]. For this reason, whole blood levels of the drug represent a more meaningful marker for its pharmacokinetics than the plasma levels. Among the different cell types, the drug is largely accumulated in tissue macrophages which are ubiquitous. These properties represent the basis of the apparently large volume of distribution of the drug. Of interest for COVID-19 therapy, CQ/HCQ has been calculated to accumulate in the lungs.

The endosomal therasurization of the aminoquinolines also represents a basis for their slow excretion. CQ/HCQ is maintained within the body for prolonged periods after its suspension. For example, HCQ has a half-life of 2963 hours [68]. Clearance to the extracellular environment of CQ and HCQ is by exocytosis and / or through the action of the multi-drug resistance protein MRP-1, a cell surface drug transporter belonging to the ATP-binding cassette family, which also includes P-glycoprotein[37,69]. HCQ is metabolized in the liver into three active metabolites, desethylchloroquine, desethylhydroxychloroquine, and bisdesethylhydroxychloroquine[70]. Desethylchloroquine possess anti-Zika virus activity [71]. All the N-dealkylated metabolites have been implicated in heart failure and retinopathy, due to long-term treatment with chloroquine[72]. Chloroquine and desethylchloroquine concentrations decline slowly, with elimination half-lives of 20 to 60 days[73]. CQ clearance is by the renal route, 38% of the administered dose is eliminated without changes[74] .

Use of CQ / HCQ in SARS-CoV-2 infection

In vitro studies

CQ has been shown to inhibit the replication of SARS-CoV-1 in HRT-18 cells, in addition to preventing death induced by human coronavirus OC43 in newborn mice; that is, protection is achieved by the transplacental route or by means of breast milk[75]. The anti-coronaviral activity of CQ has been reported in the human fetal lung

cell line, L132, infected with HCoV-229E; in this scenario, CQ significantly decreased viral replication at lower concentrations well within the range reported in blood during in clinical use[76]. In a study with BHK-21 cells infected with recombinant virus rHCoVs-OC43 labeled with Renilla luciferase, CQ inhibited the replication of HCoV-OC43 in vitro[77].

There are three in vitro studies of the activity of CQ or HCQ against SARS-CoV-2 using Vero E6 cells infected with this virus[4,78,79]. Yao et al. compared the antiviral activity of CQ and HCQ against SARS-CoV-2, using a physiological pharmacokinetic model methodology that allowed simulating five different dosing regimens, with the aim of predicting the safest dose of these drugs. The in vitro model showed that HCQ (EC 50 = 0.72 μM) is more potent than CQ (EC 50 = 5.47 μM). Based on the study results, they would recommend administering a loading dose of 400 mg twice daily of HCQ sulfate orally, followed by a maintenance dose of 200 mg twice daily for 4 days for SARS-CoV-2[79].

Wang et al. studied the antiviral activity of CQ in Vero E6 cells (ATCC-1586) infected with SARS-CoV-2 (half-maximal effective concentration, EC50 = 1.13 μM; CC50 > 100 μM, selectivity index SI > 88.50). The EC 90 (90% effective concentrations) value of CQ against SARS-CoV-2 in Vero E6 cells was 6.90 μM; therefore, it is possible to reach an adequate concentration for clinical use, as demonstrated in plasma of patients with rheumatoid arthritis who received administration of 500 mg[78].

Liu et al. studied the in vitro anti-SARS-CoV-2 activity of HCQ using VeroE6 cells from green monkey kidney (ATCC-1586), finding that it efficiently inhibits SARS-CoV-2 infection[4].Additionally, the study confirmed that HCQ inhibits the entry of SARS-CoV-2 into cells, as well as the stages after SARS-CoV-2 entry; and CQ had similar effects[4].

Human clinical studies

The results of a number of clinical trials [80–84] and observational studies [85–99] have been reported so far, many of which presenting methodological limitations, due to duress conditions during a the conditions due to an unexpected pandemics (Table 1). Two studies also suffer from poor reporting, with no dosage being

declared [87,93] and one of them including in the HCQ arm patients with worse baseline characteristics than the control group[93].

Among the trials reporting the dosages adopted, the results are reminiscent of those reported in the context of HIV/AIDS, another disease in which CQ/HCQ use was postulated to be beneficial because of both reported antiviral activity and inhibition of immune activation [100], showing dose dependency of the positive outcomes.

Seven of the COVID-19 clinical studies were conducted with a median dosage of 400 mg/day of HCQ, with or without a loading dose and an association with azythromycin. Two of these [including one randomized clinical trial (RCT)] resulted in positive outcomes and five (again, including one RCT) report negative results. One of these studies, though, reported results comparing the use of HCQ with that of another antiviral agent (lopinavir/r) [99]. Among the two observational studies conducted with the same dosage preceded by a loading dose (LD) resulted in opposite results, with the trial using the higher LD (800 mg/day) being the one reporting positive results. Among the two observational studies conducted with the same dosage preceded by a loading dose (LD) resulted in opposite results, with the trial using the higher LD (800 mg/day) being the one reporting positive results [91]. Among the studies using 600 mg of HCQ daily, four reported positive outcomes and three did not. Five of these studies were only observational (three with positive and two with negative results)[86,90,96,97,101]. Some of the studies using daily 600mg HCQ studies associated HCQ with azythromycin apart from an observational study which showed negative results.[82,94,102].

One RCT of HCQ using an LD of 1200 mg on the first day followed by 800 mg of the drug daily had a negative outcome [80].This dosage of HCQ is slightly lower than the maximum dosage administered to patients with autoimmune diseases. This trial, however, was biased by the background antiviral therapy. In the first version of the clinical trial that the authors filed [80] the authors showed that, after stratification of the patients by background antiviral therapy, the use of HCQ decreased the risk for hospitalization. The reason why the authors removed this analysis in the subsequent version of the study is unclear [80]. As of May 17 2020, the study has not yet been peer-reviewed. Both articles reporting results on CQ (1000 mg daily), state that there was a positive outcome in terms of virus negativization[85,95]. Finally, one study[103] reports results of a trial including two arms, one of which treated with the maximum dosage of CQ so far administered to humans (1200 mg daily). The trial was interrupted because of significant toxicity resulting in increased number of deaths.

Another recent study merits to be dealt with in particular detail because of the level of alarm raised through its large media coverage and the elevated number of people on whom it was conducted[101]. After conducting a retrospective analysis on 671 hospitals in six continents, Mehra et al. conclude that CQ and HCQ, particularly in combination with macrolide antibiotics, increase the number of deaths in hospitalized patients with COVID-19 and that this excess mortality is associated with increased arrhythmias. The study, however, is biased by non-homogeneous distribution of pre-existing risk factors. For example, the treatment groups had higher incidence of current cigarette smoking, hypertension, and a larger body mass index (BMI), all factors in general associated with poorer prognoses. Some of these factors such as hypertension or BMI resulted to be independent predictors of mortality according to the analyses done by the same authors. Although none of these factors was significantly higher in the CQ/HCQ groups, it cannot be excluded that their cumulative association in these groups may have been the fatal determinant for increased mortality. Moreover, it is not clear why only patients treated with remdesivir but not those treated with any of the other antivirals were excluded from the analysis. There were background antiviral interventions, and the distribution of the different antivirals in the CQ/HCQ, non-CQ/HCQ groups is not reported. It is known that some antivirals such as lopinavir/r, when administered at full dosages can increase the incidence of arrhythmias [104] and this analysis should therefore have been reported. Finally, the study filed to detect the contribution of cigarette smoking to the incidence of arrhythmias, an association which is largely documented in literature [105]. Despite these limitations, the study supports the notion of cautious monitoring of patients receiving chloroquine/hydroxychloroquine, in particular those who have independent risk factors potentially associated with higher mortality from COVID-19.

The toxicity profile thus showed a pattern similar to that observed with the positive outcomes, with higher numbers of events observed with the highest dosages of CQ/HCQ. The study of Borba et al.[103] administering the highest CQ dosage, however, is biased by the fact that the authors administered such a high dosage of CQ concomitantly with azithromycin, for reasons that will be apparent below. In general, the results so far obtained can be explained by recent calculations taking into account the pharmacokinetics of CQ/HCQ. Taking into account the mathematical model developed by Goncalves et al. [106] recent pharmacokinetic analyses [107] and some immune modulating properties of the drug [108]. Tarek and Savarino calculated that CQ/HCQ may have a limited impact on viral clearance, being evident only within a narrow window of tissue concentrations immediately below those causing toxicity [109]. The results of this modeling study also highlight a problem

underlying many of the aforementioned clinical studies, which were conducted in patients already hospitalized: an antiviral effect of HCQ is to be expected when the drug is administered immediately early after diagnosis, before patients are hospitalized.

Finally, in regard of very early administration, a recently published study shows a potential for HCQ as a post-exposure prophylaxis [110]. The study reports on a post-exposure prophylaxis regimen that was conducted in 211 patients and health workers following exposure to two infected healthcare workers. After a median period of 10 days of preventive treatment with hydroxychloroquine (400 mg / day), nobody tested positive for the virus. Unfortunately there was no control group. The results of a controller clinical trial of HCQ prophylaxis will soon be available [111].

Adverse drug reactions

Adverse drug reactions (ADRs) related to CQ/HCQ can be generally divided into two types, depending on the duration of the administration. The first type of ADR occurs when administered for a short time (<1 month), as in the treatment or prophylaxis of malaria ("acute toxicity"). The second type of ADR appears when it is administered for long periods of time (years), as occurs in the treatment of systemic lupus erythematosus and rheumatoid arthritis, and produced by accumulation of the drug in the body (“cumulative toxicity”) [112]. Both types of CQ/HCQ-induced ADRs have been extensively studied, for more than 50 years, as literally hundreds of tons of the drug have been administered to more than 200 million malaria patients [113]. Severe but very rare ADRs have been observed when administered for several years and occur due to the accumulation of the drug in the body.

Short-time safety considerations

Regarding the safety of CQ / HCQ and its administration schedules for SARS-CoV-2, it is possible to make a comparison with acute compare it with that reported during administration in the treatment of malaria. For SARS-CoV-2 treatment, a duration of 5 to 20 days has been recommended according to the severity of the case, with a maximum dose of 1000 mg / day of CQ, or the equivalent of HCQ. In the treatment of malaria, the dose is 25mg / kg for 3 days (in a 60 kg patient, 1500mg / day) [114]. The most frequent CQ / HCQ ADRs when administered for malaria are pruritus (6-50.9%), dizziness (9.6-22.69%), vomiting (1-15.8%), abdominal pain (2-13.3%), headache (9.6-13.2%), insomnia (9.6%), nausea (6.53-11.3%), and asthenia (5.3-9.6%)[115–118]. The

most serious but very rare ADRs have been reported in treatment for more than 5 years, among the two most important being cardiotoxicity and retinopathy. Cardiotoxicity during treatment for malaria is very rare; clinically relevant prolongation of the QTc interval has been observed; and no cases of retinopathy have been reported when administered for this indication[119]. Reported cases of severe arrhythmias (*torsades de pointes*) or sudden death have been reported in patients on more than 5 years of treatment due to autoimmune diseases[120].

Safety concerns have been raised for cardiac toxicity also during acute treatment with HCQ [121]. In this regard, important insight on safety issues can be derived from a recent survey on data from almost one million patients with autoimmune disease treated with HCQ [122]. The results show that there is no risk for significant prolongation of the QT interval in patients treated with HCQ alone for less than 30 days in comparison with those treated with sulfasalazine. On the other hand, the risk was increased when HCQ was used in combination with azithromycin.

It may be argued that, because COVID-19 causes cardiac problems, the cardiac toxicity of HCQ can be enhanced also in the short term. These considerations can be rejected in light of the fact that also autoimmune diseases such as lupus and rheumatoid arthritis for which HCQ has been used for decades, can affect the heart. Moreover, a number of guidelines have been issued to prevent an circumvent HCQ-related cardiac toxicity in patients with COIVD-19 [121,123,124]; would be highly recommended at this stage.

It has been hypothesized that also a short HCQ treatment might be detrimental in the treatment of COVID-19, because the drug may impair innate immunity and thus deprive the organism from an important weapon of self-defense against the virus [125]. These considerations however are only theoretical and seem not to be applicable in the context of treatment of an acute infectious disease such as COVID-19. First, an investigation conducted on a large number of patients treated with HCQ for lupus erythematosus showed that in fact the drug decreases the infectious events [126]. Second, the HCQ analogue CQ was shown to significantly increase cell-mediated responses in response to a viral antigen [108,127]. Cell-mediated responses have recently been shown to play a major part in protection against SARS-CoV-2 in vivo [128]

Chronic treatment safety issues

In a systematic study on chronic use (3.25 to 7.9 years) of CQ/HCQ in patients with systemic lupus erythematosus, HCQ had fewer adverse reactions than CQ. The proportions of ADRs were nausea (7-12%),

diarrhea (18%), myopathy (1.3%), headache (1.3% -12%), ototoxicity (0.6%), and dermatological such as urticaria (0.6 % -12%)[119]. The frequency of cardiotoxicity such as conduction disorders (0-4%) and cardiomyopathy (0-1.3%) were very rare[129]. The frequency of retinal toxicity ranged from 0.33% to 16%, and a study compared the frequency between CQ and HCQ (19% vs. 0%) [130].

CQ / HCQ-induced cardiotoxicity is related to certain risk factors such as advanced age, female sex, prolonged duration of therapy (> 10 years), high daily dose per kilogram, pre-existing heart disease and kidney failure[131]. Chatre et al. conducted a systematic study on cardiotoxicity associated with CQ / HCQ; of the total cases, 15% were patients on short-term treatment (malaria), and the remaining were patients on prolonged treatments for connective tissue diseases[112]; they found that cardiotoxicity was predominant in women (65%); the mean use of CQ / HCQ was 7 years (range 3 days to 35 years), higher in CQ users than HCQ, and the mean cumulative dose was 1235 g for HCQ and 803 g for CQ. The most common CQ / HCQ-induced cardiac disorder was conduction disorders (85%), among which are in order of frequency atrioventricular block, first and second degree block, complete AV block, right bundle branch block, and left bundle branch block. Other non-specific adverse cardiac events included ventricular hypertrophy (22%), hypokinesia (9.4%), heart failure (26.8%), pulmonary arterial hypertension (3.9%), and valve dysfunction (7.1%).In 78 (61%) patients the medication was withdrawn and 44.9% recovered normal cardiac function; 12.8% of ADRs persisted and mortality was 30.8%. It is important to emphasize that this systematic study reviewed cases of cardiotoxicity in more than 40 years of CQ/HCQ use in the world (the study covers reports from 1975 to 2017)[112].Acute cardiotoxicity occurs due to alteration in ion channels with a destabilizing effect on the membrane, increased QT interval, a negative inotropic effect and atrioventricular block. On the other hand, cumulative cardiotoxicity occurs by accumulation of the drug in the body, which increases lysosomal pH, with alteration of lysosomal protein degradation, accumulation of autophagosomes, phospholipids, and glycogen with vacuolization of myocytes[112].

Keratoplasty and retinopathy induced by CQ / HCQ has not been described when used as antimalarial. The frequency is very low, and they have been described in patients who used HCQ for more than 10 years and at high dose [132]. The Incidence of HQC retinopathy is 0.4% in patients whose daily dosage is >6.5 mg/kg or who have taken HCQ continuously for > 10 years[133–135]. Bilateral pigmentary retinopathy induced by CQ / HCQ

begins with subtle paracentral scotomas, followed later by "bull's-eye" maculopathy, which is characterized by a ring of retinal pigment epithelium (RPE) in the macular area closest to the fovea and the final stage with generalized RPE and atrophic retina with loss of central, peripheral and night vision. Risk factors for CQ retinopathy are doses greater than 2.3 mg / kg and HCQ > 5.0 mg / kg, duration of therapy greater than 5 years, kidney failure, drug interaction (e.g. Tamoxifen), and previous macular disorders that make it difficult note the changes in the follow-up eye exams [120,136].

Precautions in the use of CQ / HCQ in patients with COVID-19

Currently, CQ / HCQ are considered safe drugs for indications of malaria and for prolonged use in certain autoimmune diseases; however, in the context of COVID-19 use, especially in the most severe forms of presentation, precautions must be taken, which are listed in table A (supplementary file). The Liverpool Drug Interaction Group (based at the University of Liverpool, UK), in collaboration with the University Hospital of Basel (Switzerland) and Radboud UMC (Netherlands), have produced various materials in PDF format to aid the use of experimental agents in the treatment of COVID-19: https://www.covid19-druginteractions.org/

Clinical Practice Guidelines in anti-SARS-CoV-2 antiviral therapy including CQ / HCQ

Currently there are fourteen on-line accessible clinical practice guidelines based on expert consensus, in the following countries: Belgium, USA (2), China (3), Ireland, Italy (2), France, Spain, Ecuador and Iran:

a) Belgium: The Dutch Center for Disease Control suggested prescribing HCQ in COVID-19 positive patients. It is not indicated in suspected cases, even with risk factors. The duration of administration of HCQ is according to severity, from 5 to 10 days. In severe cases it suggests administration of HCQ by nasogastric tube. On the 5th day, adverse reactions should be evaluated considering the long half-life (30 hours) [137].

b) China, Zhejiang University School of Medicine: The guideline suggested administering CQ in COVID-19 positive patients, only if the basic regimen is not effective (lopinavir / ritonavir, combined with arbidol) [138]

c) China, Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for Chloroquine in the treatment of novel coronavirus pneumonia: The indication for CQ administration is the diagnosis of pneumonia in COVID-19 positive patients over 18 and under 65 years of age. The consensus suggests administering Chloroquine phosphate, 500 mg each time, 2 times / day for 10 days. If severe gastrointestinal reactions occur, the dose may be reduced to 1 time / day, 500 mg each day, or even discontinued. During the treatment course, if the test for throat swab coronavirus becomes negative and negative for 3 days, withdrawal of the drug may be considered, but the minimum course of treatment is 5 days. Precautions during treatment with QC include monitoring with pharyngeal swabs during treatment, full blood count, cardiac enzymes every 2 days,electrocardiogram before and after starting the drug (day 5 and 10) and evolution of the clinical picture with chest CT [139][76].

d) Ireland, HSE National Clinical Advisor and Group Lead, Acute Hospitals: suggest administration of CQ or HCQ to all confirmed patients with COVID-19 infection.[140]

e) Italy, National Institute for Infectious Diseases, “L. Spallanzani”, IRCCS: suggested administration of HCQ associated with base therapy (e.g. Lopinavir / Ritonavir) in all confirmed patients with symptomatic COVID-19, lasting 10 days [141].

f) Italy, Italian Society of Infectious and Tropical Diseases SECTION Regione Lombardia, suggests administering CQ or HCQ to all patients confirmed with COVID-19, over the age of 70 and / or with risk factors, and / or symptomatic. The duration of the treatment can be from 5 to 20 days according to the severity of the pneumonia. In severe cases, it suggested administering HCQ by nasogastric tube.[142]

g) COVID-19 Management Guidelines, Pakistan Chest Society, suggests administering HCQ loading dose 400 mg bid then 200 mg tid for 10 days or Chloroquine 500mg bid x 10 days. [143]

h) USA, UW Medicine suggested administering HCQ in confirmed with COVID-19, with risk factors and over 60 years. with a duration depending on the severity of the case, from 5 to 10 days [144].

i) France, SRLF-SFAR-SFMU-GFRUP-SPILF, Misson COREB Nationale. CQ is recommended at 500 mg twice a day. Alternatively, HCQ was recommended at 200 mg, three times a day. This dosage is higher than that recommended in other clinical guidelines, such as the Italian; yet the dosage of HCQ is not enough as to match the equivalent dosage to 1000 mg/day of CQ, which would be 800 mg/day of HCQ, as based on studies in antimalarial treatment.[145]

j) Spain, Agencia Española de Medicamentos y Productos Sanitarios (AEMPS). In adults, the recommended dose of HCQ is 400 mg twice daily on day one followed by 200 mg twice daily for the rest of the course (5 days). Alternatively, CQ 620 mg followed by 310 mg twelve hours later on day one, followed by 310 mg twice daily for the rest of the course (5 days).[146]

k) Ecuador, Ministry of Public Health, Therapeutic Guide for COVID-19.CQ/HCQ is indicated in hospitalized patients (ICU or ward).[147]

l) Iranian Expert's Consensus Statement, Algorithmic Approach to Diagnosis and Treatment of Coronavirus Disease 2019 (COVID-19) in Children: suggest use of QC associated with other antivirals in for patients who admitted in intensive care unit, combined antiviral agents and immunomodulators.[148]

On March 28, 2020, FDA authorized use of QC/HCQ to treat adult and adolescent patients who weigh 50 kg or more hospitalized with COVID-19 and for whom a clinical trial is not available, or participation is not feasible (https://www.fda.gov/media/136534/download). FDA however changed the guidelines after a while. Due to toxicity issues emerging, they then recommended that CQ/HCQ be not prescribed outside the hospital setting or the context of registered clinical trials [149]

The Italian Drug Agency (AIFA), having first authorized CQ/HCQ treatment also for non-hospitalized COVID-19 patients [150], has stopped recommending the use of CQ/HCQ for treatment of COVID-19 [151], following the aforementioned report of Mehra et al. [101]. Following the same report [101], also France has stopped recommending the use of CQ/HCQ[152]. The Spanish drug regulatory agency has instead decided to maintain the recommendation for HCQ treatment, due to the limitations of the aforementioned report [153].

Conclusion

In the current context of the SARS-CoV-2 pandemic, with disastrous health and economic consequences, it is important to consider all the strategies to combat it, in relation to drug selection, which will always be based on their efficacy and safety. There has been significant research on the possible antiviral action of CQ / HCQ. Their safety aspects have been studied extensively for over 50 years, but the evidence is not necessarily applicable to those most at risk of mortality from Covid-19 (e.g. frail older people), who at the same time are most vulnerable to drug side effects. The challenge that SARS-CoV-2 launches into science is to create new specific drugs. However, in the meantime further research on the possible benefits/risks of CQ / HCQ is an appropriate step forward. Subject to a still favorable risk/benefit balance, CQ / HCQ could become part of the pharmacological armamentarium in the war against SARS-CoV-2.

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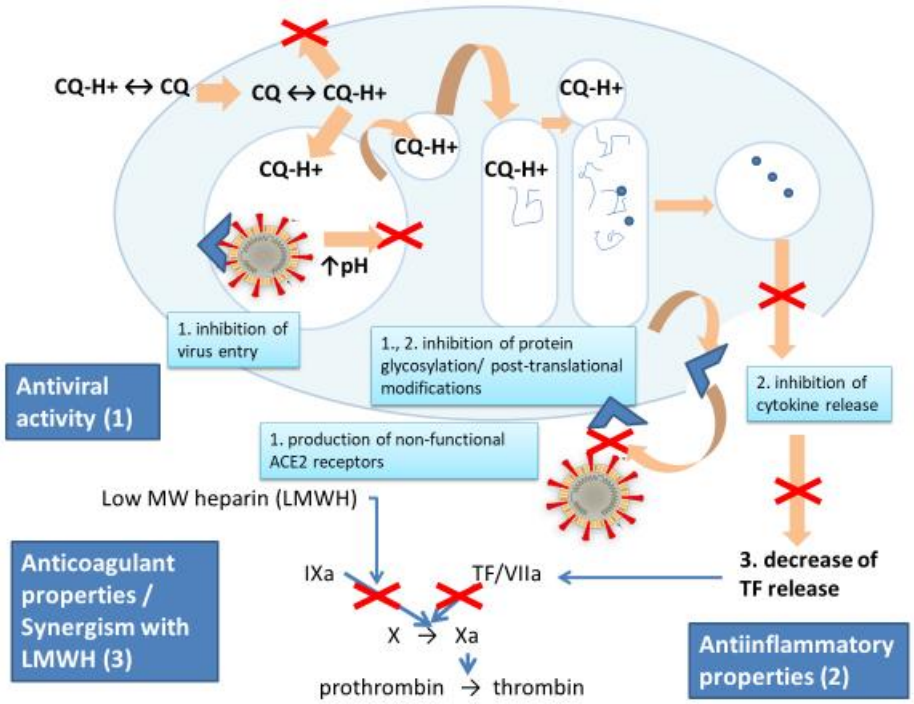

Figure 1. Chloroquine (CQ) and hydroxychloroquine (HCQ): Specific Anti-SARS-CoV-2 potential mechanisms of action

1003 Supplementary file

1004 Table A

1005 Precautions during CQ/HCQ administration as treatment for COVID-19*.

System/tissue	Potential side effects	Monitoring
Heart	• QT interval prolongation, torsade de pointes and ventricular arrhythmias: use with caution in patients with a history of such disorders, in patients with uncorrected hypokalaemia and / or hypomagnesemia, or bradycardia (HR <50 beats per minute). • Avoid concomitant use of drugs that prolong QTc. • Recommended monitoring for signs and symptoms of cardiomyopathy due to cases that have resulted in heart failure (some fatal).	ECG: QTc prolongation may occur. Use with caution if pre-existing QTc prolongation and/or known risk factors for prolongation of the QTc interval (including concomitant administration of other QTc prolonging agents).
Diabetes / metabolic	• Hypoglycaemia: monitoring is recommended due to cases of severe hypoglycaemia can be fatal, in patients treated or not with antidiabetics. • Insulin requirements may decrease.	Blood glucose: may cause hypoglycaemia.
Neurological	• Risk of decreased epileptic threshold: caution in patients with epilepsy or seizures and / or when used concomitantly with other drugs that lower the epileptic threshold. • Extrapyramidal reactions: Caution in case of Parkinson's disease (mentioned as a contraindication).	
Eyes and retina	• Retinopathy / maculopathy: If vision disturbance indicating retinopathy / maculopathy is observed during treatment, chloroquine should be discontinued immediately, and the patient should be observed due to the risk of possible progression. • Avoid concomitant use of medications that can affect the retina: such as tamoxifen. • Changes in the retina (and visual disturbances) can still progress even after stopping therapy. • Although the risk of retinopathy / maculopathy is greater in the case of long-term treatment, since the damage may be irreversible, it is prudent to recommend an ophthalmic examination.	Retinal toxicity: Due to low risk with recommended dose and duration of treatment, ophthalmological examination not required in context of COVID-19 infection.
Renal	• Caution in patients with kidney failure.	CrCl 30-50mL/min: 75% of dose CrCl 10-30mL/min: 25-50% of dose. CrCl<10mL/min: 25-50% of dose. CVVHD (Continuous Venous-Venous Haemodialysis): 25-50% of dose. Recommend using upper dose range in context of COVID-19 infection.

		Extending dose intervals rather than dose reductions may be necessary for practical reasons.
Haematological. Glucose-6-phosphate dehydrogenase (G6PD) deficiency.	• Risk of methemoglobinemia / haemolytic anaemia in patients with glucose-6-phosphate dehydrogenase deficiency. • Recommend obtaining G6PD test. Post-marketing studies suggest the risk of haemolysis is very low. It is reasonable to start hydroxychloroquine in most patients while awaiting G6PD testing.	Full Blood Count: Myelosuppression may occur rarely; monitor if pre-existing myelosuppression or if receiving other myelosuppressive agents concomitantly. G6PD: Caution advised in patients with G6PD deficiency, may be risk of haemolysis. If status unknown, do not delay initiation of treatment in the context of moderate or severe COVID-19.
Gastrointestinal	• GI symptoms can be mitigated by taking hydroxychloroquine with food.	
Others	• Caution in patients with liver failure. • Caution in patients with intermittent porphyria, taking chloroquine can induce an acute attack. • Exacerbations of psoriatic lesions in patients with psoriasis	

Adapted from:

1)van Iersse S DN, Bottieau E, Huits R. . Interim clinical guidance for patients suspected of/confirmed with covid-19 in Belgium - 06 May 2020; Version 8. Available on: https://covid-19.sciensano.be/sites/default/files/Covid19/COVID-19_InterimGuidelines_Treatment_ENG.pdf

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Table

Table 1

Studies on the effectiveness and safety of chloroquine (CQ) and hydroxychloroquine (HCQ) in SARS-CoV-2 infection

Author	Institution /Country Study Conducted	Study design	No. Patients	treatment regimen/ duration (days)	Results		Adverse reactions	authors' conclusions
					Primary Outcome	Secondary Outcome		
Tang et al.(2020)	16 Chinese government designated COVID 19 centers in 3 provinces (Hubei, Henan, Anhui). China	Open label, RCT, Intention-to-treat Analysis	150	HCQ 1200mg LD D1-D3, 800mg D4 up to D14 for mild/moderate symptoms HCQ 1200mg LD D1-D3, 800mg D4 up to D21 for severe symptoms + Standard of care (included use of antivirals)	-The negative conversion probability by 28 days in SOC plus HCQ group was 85.4% (95% confidence interval (CI) 73.8% to 93.8%), similar to that in the SOC group 81.3% (95%CI 71.2% to 89.6% p>0.05)). Between–group difference was 4.1% (95%CI –10.3% to 18.5%).	The probability of symptoms alleviation by 28 days was similar between patients with SOC with (59.9%, 95%CI 45.0% to 75.3%) and without HCQ (66.6%, 95%CI 39.5% to 90.9%, p>0.05)). -The median time to alleviation of clinical symptoms: SOC plus HCQ group vs SOC group (19 days *versus* 21 days, Hazard ratio, 1.01, 95%CI, 0.59 to 1.74, p=0.97 by log–rank test)	Diarrhea: 10% Blurred vision: 1.4% (transient with a period of 1–2 days)	The administration of HCQ did not result in a significantly higher negative conversion probability than SOC alone in patients mainly hospitalized with persistent mild to moderate COVID–19. Adverse events were higher in HCQ recipients than in HCQ non–recipients
Cheng Z. et al. (2020	Renmin hospital of Wuhan University in Wuhan, China	Double blind, RCT, Intentionto-treat analysis	62	HCQ 400mg D1-D5 + standard ofCare	-Time to clinical recovery (TTCR), TTCR, the body temperature recovery time and the cough remission time were significantly shortened in the HCQ treatment group. - Patients progressed to severe illness in control and QCH groups: 4/31 (12.9%) vs 2/31 (6.45%).	-Absorption of pneumonia on chest CT, control vs HCQ group: 17 (54.8%) vs 25 (80.6%). -Fever in control and QCH groups: days (SD) 3.2 (1.3) vs 2.2 (0.4) p= 0.0008); Cough, day (SD) 3.1 (1.5) vs 2.0 (0.2) p=0.0016.	-Control vs HCQ group: 0% vs 6.4 % (rash, headache)	Among patients with COVID-19, the use of HCQ could significantly shorten TTCR and promote the absorption of pneumonia.
Chen Jun et al. (2020)	Shanghai Public Health Clinical Center in Shanghai, China	Open label, RCT, Intentionto-treat analysis	30	HCQ 400mg D1-D5 + standard ofcare	On day 7, COVID-19 nucleic acid of throat swabs was negative in 86.7% cases in the HCQ group and 93.3% cases in the control group ($P > 0.05$).	Radiological progression was shown on CTimages in 5 cases (33.3%) of the HCQ group and 7 cases (46.7%) of the control group, and allnpatients showed improvement in follow-up examination.	Four cases (26.7%) of the HCQ group and 3 cases (20%) of the control group had transient diarrhea and abnormal liver function ($P > 0.05$)	The prognosis of common COVID-19 patients is good. Larger sample size study are needed to investigate the effects of HCQ in the treatment of COVID-19.
Gautret et al (RCT) (2020)	University Hospital Institute Méditerran é Infection in Marseille, France	Open label, nonrandomize d clinical trial, Perprotocol analysis	42	HCQ 600mg D1-D10 ± Azithromycin 500mg LD, 250 mg D2-D5 + Standard of care	At day 6 post-inclusion, 70% of HCQ treated patients were virologically cured as compared 12.5% in the control group (p= 0.001)	Drug effect was significantly higher in patients with symptoms of URTI and LRTI, as compared to asymptomatic patients with p<0.05 URTI: upper tract respiratory infection, LRTI: lower tract respiratory infection	No data	Despite its small sample sizethe survey shows that hydroxychloroquine treatment is significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by azithromycin.

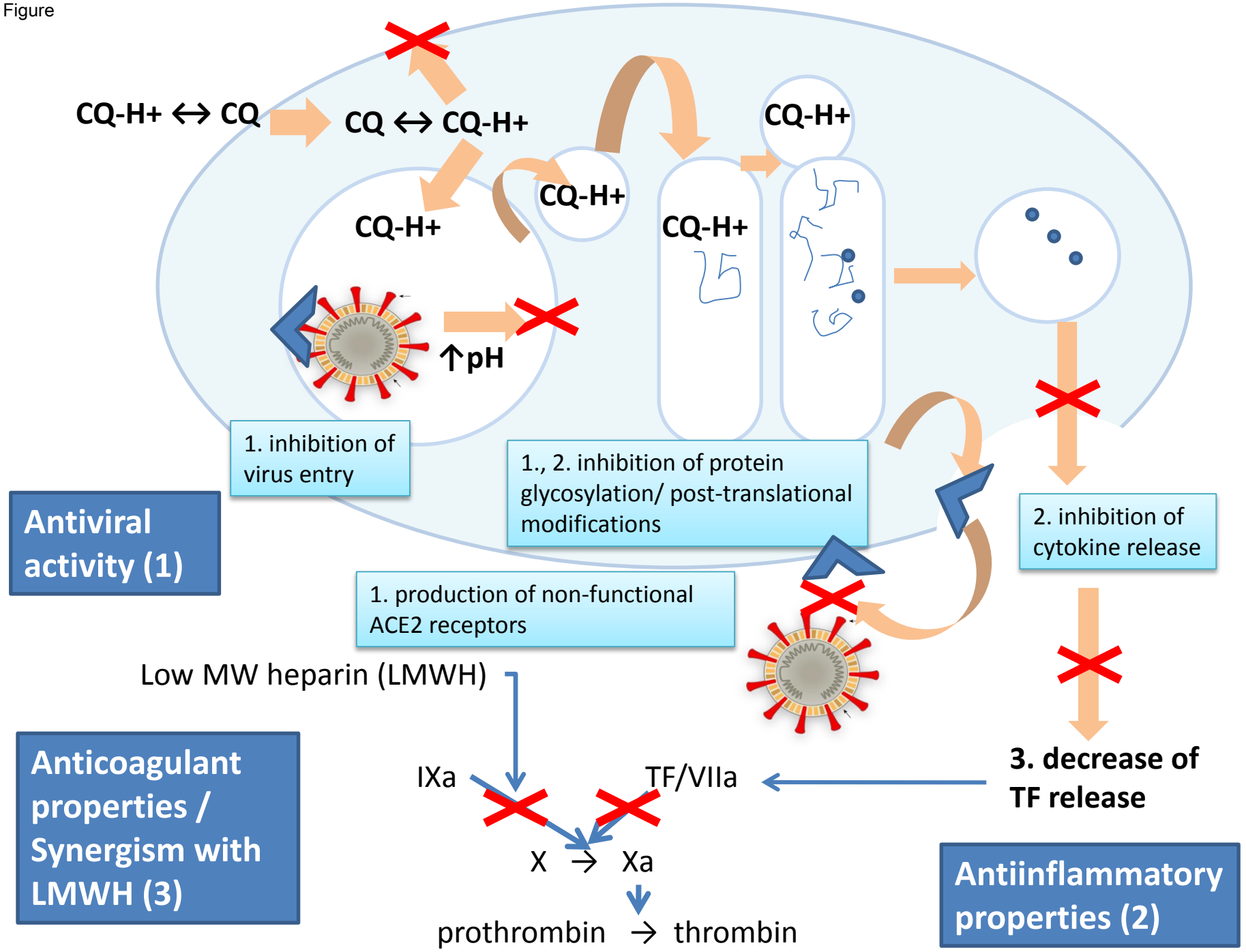
Borba M, et al (2020)	*Hospital e Pronto-Socorro Delphina Rinaldi Abdel Aziz*, in Manaus, Western Brazilian Amazon	double-blinded, randomized, phase IIb clinical tr	440	CQ (600mg CQ twice daily for 10 days or total dose 12g); or low dose CQ (450mg for 5 days, twice daily only on the first day, or total dose 2.7g).	The high dosage CQarm presented more QTc>500ms (18.9%), and a trend toward higher lethality (39%) than thelower dosage. Fatality rate until day 13 was 27% (95%CI=17.9-38.2%), overlapping with theCI of historical data from similar patients not using CQ (95%CI=14.5-19.2%).	In 27 patientswith paired samples, respiratory secretion at day 4 was negative in only six patients (22%).	The high dosage CQ arm presented more QTc>500ms (18.9%), and a trend toward higher lethality (39%) than the lower dosage.	Preliminary findings suggest that the higher CQ dosage (10-day regimen) should not be recommended for COVID-19 treatment because of its potential safety hazards
Huang Mingxing et al. (2020.).	12 hospitals in Guangdong and Hubei Provinces.China	multicenter prospective observational study	197	CQ 500mg, orally,twice (half dose) or once(full dose) daily. D1-D10	The median time to achieve an undetectable viral RNA was shorter in CQ than in non-chloroquine(absolute difference in medians -6.0 days; 95% CI -6.0 to -4.0 $P < 0.0001$)	-The duration of fever is shorter in CQ (geometric mean ratio 0.6; 95% CI 0.5 to 0.8; P =0.0029;). - There are 1/197 (=.51%) patient in the CQ group experiencedaggravated symptoms from moderate to severe, while 9/ 176(5.11%) patients in the non CQ group have the same aggravated experience	Any adverse events CQ vs non chloroquine group: 26.9% vs 32.4%). -Vomiting: 4.6% vs 1.1% -Nausea: 9.1% vs 4% -Dizziness: 1.2 vs 2.3% Blurred vision: 1.5% vs 0%. -Ventricular premature beat: 0 vs 0.6%	Evidence for safety and efficacy of CQ in COVID-19
Million et al . (2020)	Assistance Publique-Hôpitaux de Marseille (AP-HM), Southern France in the InstitutHospitalo-Universitaire (IHU) *Méditerranée Infection. France.*	observational study	1061	HCQ (200 mg three times daily for ten days) + AZ (500 mg on day 1 followed by 250 mg daily for the next four days) for at least three days.	-Good clinical outcome and virological cure were obtainedin 973 patients within 10 days (91.7%).	A poor clinical outcome (PClinO) was observed for 46 patients (4.3%) and 8 died (0.75%) (74-95 years old).All deaths resulted from respiratory failure and not from cardiac toxicity.	mild adverse events: 2.3% (gastrointestinal or skin symptoms, headache, insomnia and transient blurred vision).	Administration of the HCQ+AZ combination before COVID-19 complications occur is safe and associated with very low fatality rate in patients
Yu B. et al (2020)	Tongji Hospital, Wuhan, China	observational study	568	HCQ 200 mg twice a day for 7-10 days	-Mortalities are 18.8% (9/48) in HCQ group and 45.8% (238/520) in Non-HCQ group ($p<0.001$). -	The level of inflammatory cytokine IL-6 was significantly lowered from 22.2 (8.3-118.9) pg/mL at the beginning of the treatment to 5.2 (3.0-23.4) pg/ml ($p<0.05$) at the end of the treatment in the HCQ group but there is no change in the NHCQ group.	No data	Hydroxychloroquine treatment issignificantly associated with a decreased mortality in critically ill patients with COVID-19 through attenuation of inflammatory cytokine storm. Therefore, hydroxychloroquine should be prescribed for treatment of critically ill COVID-19 patients to save lives.
Mallat, J. et al. (2020)	Cleveland Clinic Abu Dhabi	Retrospective observational study	34	HCQ 400 mg was administered twice daily for 1 day, followed by 400 mg daily for 10 days.	The time to SARS-CoV-2 negativity test was significantly longer in patients who received HCQ compared to those who did not receive the treatment (17 [13-21] vs. 10	No patients were admitted to intensive care unit, required high flow oxygen therapy, non-invasive or invasive mechanical ventilation, and all of them were discharged alive from	HCQ was well tolerated with no observed side effects	HCQ was associated with a slower viral clearance in COVID-19 patients with mild to moderate disease.

					[4-13] days, p=0.023,	the hospital.		
Magagnoli et al. (2020)	data from patients hospitalized with confirmed SARSCoV-2 infection in all United States Veterans Health Administration medical centers until April 11, 2020.	Retrospective observational study.	368	exposure to HCQ alone or with azithromycin (HCQ+AZ) as treatments in addition to standard supportive management for Covid-19.	Compared to the no HCQ group, there was a higher risk of death from any cause in the HC group (adjusted HR, 2.61; 95% CI, 1.10 to 6.17; P=0.03) but not in the HC+AZ group (adjusted HR, 1.14; 95% CI, 0.56 to 2.32; P=0.72) -Important: baseline Pulse oximetry (SpO2) > 95: HC, HCQ and No HC, 62.9 , 57.5 and 73.4% respectively. There was a higher percentage of patients with (SpO2) > 95 in those who did not receive HC/HC+AZ..	-No significant difference in the risk of ventilation in either the HC group (adjusted HR, 1.43; 95% CI, 0.53 to 3.79; P=0.48) or the HC+AZ group (adjusted HR, 0.43; 95% CI, 0.16 to 1.12; P=0.09), compared to the no HC group	No data	No evidence that use of HCQ, either with or without azithromycin, reduced the risk of mechanical ventilation in patients hospitalized with Covid-19
Molina et al (2020)	Saint Louis Hospital, Paris, France	Prospective uncontrolled single arm study	11	600mg/day of HCQ for 10 days + Azithromycin 500mg day 1 followed by 250mg/day next 4 days	Nasopharyngeal swabs in 8/10 patients were still positive for SARS-CoV2 RNA at days 5 to 6 after treatment initiation		No data	No evidence of a strong antiviral activity or clinical benefit of the combination of HCQ and azithromycin in severe ill COVID-19 patients
Gao et. al. (2020)	10 hospitals in China in cities of Wuhan, Jingzhou, Guangzhou , Beijing, Shanghai, Chingqing, Ningbo	observational study	100	CQ 500mg BID D1-D10 + Standard of Care	100 patients have demonstrated that chloroquine phosphate is superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus-negative conversion, and shortening the disease course according to the news briefing.		Severe adverse reactions to chloroquine phosphate were not noted in the aforementioned patients	CQ, is shown to have apparent efficacy and acceptable safety against COVID-19 associated pneumonia
Gautret et al (OS) (2020)	University Hospital Institute Méditerran é Infection in Marseille, France	observational study	80	HCQ 600mg D1-D10 + Azithromycin 500mg LD, 250mg D2-D5	Nasopharyngeal viral load 83% negative at Day7, and 93% at Day 8, Viral culture negativity 97.5% at Day5		Nausea or vomiting: 2.5% Diarrhoea: 5% Blurred vision:1.2%	HCQ + AZI is effective in the treatment of COVID-19.
Mahévas M. et al (2020)	French hospitals with documented SARS-CoV-2 pneumonia and requiring oxygen ≥ 2 L/min	observational study	181	HCQ at 600 mg/day	20.2% patients in the HCQ group were transferred to the ICU or died within 7 days vs 22.1% in the no-HCQ group (16 vs 21 events, relative risk [RR] 0.91, 95% CI 0.47–1.80)	2.8% of patients in the HCQ group died within 7 days, compared with 4.6% in the no-HCQ group (3 vs 4 events, RR 0.61, 95% CI 0.13–2.90).	ECG modifications requiring HCQ discontinuation at a median of 4 days (3-9): 9.5%	HCQ did not significantly reduce admission to ICU or death at day 7 after hospital admission, or ARDS in hospitalized patients with hypoxemic pneumonia due to COVID-19
Rosenberg et al (2020)	Inpatients admitted to	observational study	1438	HCQ 200-600mg/day.Dose and duration were variable.	there were no significant differences in mortality for patients receiving HCQ +		There were no significant differences	Among patients hospitalized in metropolitan New York with

	hospitals in the New York City (NYC) metropolitan region between March 15 and 28, 2020, USA				azithromycin (HR, 1.35 [95%CI, 0.76-2.40]), HCQ alone (HR, 1.08 [95%CI, 0.63-1.85]), orazithromycin alone (HR, 0.56 [95%CI, 0.26-1.21]		in the relative likelihood of abnormal electrocardiogram findings. Diarrhea (group HCQ+AZI=: 11.6%; HCQ alone: 17%). Hypoglycemia (group HCQ+AZI=: 3.4%; HCQ alone: 0.5%). QT prolongation: (group HCQ+AZI=: 11.6%; HCQ alone: 14.4%).	COVID-19, treatment with HCQ, azithromycin, or both, compared with neither treatment, was not significantly associated with differences in in-hospital mortality
Geleris et al. (2020)	New York–Presbyterian Hospital (NYP)–Columbia University Irving Medical Center (CUIMC), USA	observational study	1446	HCQ (600 mg twice on day 1, then 400 mg daily for a median of 5 days);	The primary end point was the time from studybaseline to intubation or death. For patients whodied after intubation, the timing of the primary end point was defined as the time of intubation. There was no significant association betweenhydroxychloroquine use and intubation or death (hazard ratio, 1.04, 95% confidenceinterval, 0.82 to 1.32).		No data	HCQ administration was not associated with either a greatly lowered or an increased risk of the composite end point of intubation or death.
Shabrawishi M. (2020)	tertiary public hospital in Mecca, Kingdom of Saudi Arabia	observational study	93	CQ or HCQ with or without any dose of azithromycin There were three interventional subgroups (group A (n=45): who received antimalarial drug only classified as (A1), combined with azithromycin (A2) or combined with antiviral drugs (A3)), and one supportive care group (group B) (n=48	Theprimary and secondary endpoints of the study were achieving negative SARS-CoV-2nasopharyngeal PCR sample within five days or less from the start of the intervention and 12 days or less from the diagnose, respectively. In group A 73.3% (n= 33) achieved the primary endpoint and 84.4% (n= 38) achieved the secondary endpoint. Smaller percentage of patients 68.8 (n= 33) and 79.2% (n= 38) achieved the primary and secondary endpoints in group B. There was no statistically significant difference in the median time to negative conversion from the first positive to the first negative PCR sample or from the time of starting the intervention between the two groups (p>0.05)		No data	Prescribing antimalarial medications was not shown to shorten the disease course nor to accelerate the negative PCR conversion rate.
Lee J. et al (2020=	hospitals in Busan, South Korea	observational study	72	HCQ (400 mg orally every 24 hours), 7 days	Among the 72 patients with mild-to-moderate disease severity on admission, 45 received LPV/r and 27 received HCQ as their initial therapy.	Disease progression was also significantly more common in the HCQ group than in the LPV/r group (44% [12/27] and 18% [8/45],	Experienced adverse effects and LPV/r , HCQ 22 (49%); 7 (26%), respectively .	LPV/r appears to be more effective than HCQ at preventing progression to severe

					Switching therapy due to clinical failure was significantly more common in the HCQ group than in the LPV/r group (41% [11/27] and 2% [1/45], respectively, *P*=0.001).	respectively, *P*=0.030).	Drug interruption due to adverse effects LPV/r , HCQ 2 (4%) 1 (4%).	disease in patients with COVID-19.
Mehra M. et al (2020)	The registry comprised data from 671 hospitals in six continents. We included patients hospitalised between Dec 20, 2019, and April 14, 2020, with a positive laboratory finding for SARS-CoV-2.	observational study	96 032	The mean daily dose and duration of the various drug regimens were as follows: CQ alone, 765 mg (SD 308) and 6.6 days (2.4); HCQ alone, 596 mg (126) and 4.2 days (1.9); CQ with a macrolide, 790 mg (320) and 6.8 days (2.5); and HCQ with a macrolide, 597 mg (128) and 4.3 days (2.0).	After controlling for multiple confounding factors, when compared with mortality in the control group (9·3%), HQC(18·0%; hazard ratio 1.335, 95% CI 1.223–1·457), HQC with a macrolide (23·8%; 1.447, 1.368–1.531), CQ (16·4%; 1.365, 1.218–1.531), and CQ with a macrolide (22.2%; 1.368, 1.273–1.469) were eachindependently associated with an increased risk of in-hospital mortality.	Compared with the control group (0·3%), HCQ (6·1%; 2·369, 1·935–2·900), HCQ with a macrolide (8·1%; 5·106, 4·106–5·983), CQ (4·3%; 3·561, 2·760–4·596), and CQ with a macrolide (6·5%; 4·011, 3·344–4·812) were independently associated with an increased risk of de-novo ventricular arrhythmia during hospitalisation.	No data	HCQ or CQ, when used alone or with a macrolide, was associated with decreased in-hospital survival and an increased frequency of ventricular arrhythmias when used for treatment of COVID-19.
Ahmad I. et al (2020)	Residents of three long-term care facilities in New York. USA	observational study	54	Doxycycline (100 mg PO BID for 7 days) and HCQ (two regimens: i) 200 mg PO TID for 7 days or ii) 400 mg PO BID one day, then 400 mg daily for 6 days).	85% patients showed clinical recovery defined as: resolution of fever and shortness of breath, or a return to baseline setting if patients are ventilator-dependent.	A total of 11% patients were transferred to acute care hospitals due to clinical deterioration and 6% patients died inthe facilities. Naive Indirect Comparison suggests these data were significantly better outcomes than thedata reported in Morbidity and Mortality Weekly Report (MMWR, CDC, USA) (reported on March 26, 2020) from a long-term care facility in King County,Washington where 57% patients were hospitalized, and 22% patients died.	2% had a seizure and HCQ was immediately terminated.	Doxycycline -HCQ treatment in high-riskCOVID-19 patients is associated with a reduction in clinical recovery, decreased transfer to hospital and decreased mortality were observed after treatment with DOXY-HCQ.
Membrillo FJ et al. (2020)	Inpatients from Central Defense Hospital "Gómez Ulla", Madrid, Spain,	Observational study	166	Loading dose of 800 mg + 400 mg, followed by a maintenance dose of 400 mg a day	48,8 % of patients not treated with HCQ died, 22% of those treated with HCQ (p=0,002). According to clinical picture at admission, HCQ increased the mean cumulativesurvival in all groups from 1,4 to 1,8 times.	HCQ treatment was an independent predictor of lower mortality (p=0,003, 95% CI 0,012 – 0,402).	No data	In a cohort of patients hospitalised with COVID-19, hydroxychloroquine treatment with 800mg added loading dose increased survival when patients were admitted in early stages of the disease.

Figure


CQ-H+ ↔ CQ
CQ ↔ CQ-H+
CQ-H+
CQ-H+
CQ-H+
CQ-H+
↑pH
1. inhibition of virus entry
1., 2. inhibition of protein glycosylation/ post-translational modifications
2. inhibition of cytokine release
Antiviral activity (1)
1. production of non-functional ACE2 receptors
Low MW heparin (LMWH)
IXa
TF/VIIa
X → Xa
prothrombin → thrombin
3. decrease of TF release
Anticoagulant properties / Synergism with LMWH (3)
Antiinflammatory properties (2)