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A pharmacological perspective of chloroquine in SARS-CoV-2 infection: An old drug for the fight against a new coronavirus?

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic caused by 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. Moreover, their antiviral properties have been previously studied, including against coronaviruses, where evidence of efficacy has been found. In the current race against time triggered by the COVID-19 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-molecular-weight heparin, aimed at preventing acute respiratory distress syndrome (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. This review also re-examines the real information provided by some of the published alarming reports, although concluding that cardiac toxicity should in any case be stringently monitored in patients receiving CQ/HCQ.

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1. Introduction

On 31 December 2019, 27 cases of pneumonia of unknown aetiology were reported in the city of Wuhan, Hubei Province, China, that quickly spread to various countries [1,2]. On 7 February 2020, the causative agent was identified and was subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV), and the disease that it causes was named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). When we started to conduct this review on 11 March 2020, the WHO declared the outbreak of COVID-19 as a pandemic [3]; on that date, 129 775 cases of infection had been reported in 114 countries, with 4751 deaths and 68 672 people recovered. People affected by SARS-CoV-2 infection can have a wide range of respiratory symptoms including fever, shortness of breath and cough, from asymptomatic or very mild to severe pneumonia. The mortality rate until 3 March 2020 was calculated at 3.4%. Regarding vaccine development, as of 23 February 2020 there were 15 phase I clinical trials underway. On the other hand, 23 clinical trials had been registered with different antivirals, monoclonal antibodies, methylprednisolone and teicoplanin and, among these, two with chloroquine (CQ). In the Chinese Clinical Trial Registry (http://www.chictr.org.cn), six studies of CQ and hydroxychloroquine (HCQ) for the treatment of SARS-CoV-2 infection were reported to be in progress [4–6].

CQ and HCQ are antimalarials belonging 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

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properties are of interest [7]. This review aims to describe the pharmacological basis and potential therapeutic utility of CQ and HCQ in SARS-CoV-2 infection.

2. History

CQ is an antimalarial drug that was synthesised in Germany in 1934, emerging as a substitute for natural quinine, which is extracted from the bark of the *Cinchona officinalis* tree. The healing properties of the bark of this 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 Chinchón, 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 was widely used in the treatment of malaria, the appearance of CQ-resistant *Plasmodium* isolates has decreased its use in this disease [8,9]. In 1946, HCQ was synthesised from CQ and was shown to be much less toxic than CQ in animals [10].

3. SARS-CoV-2

Coronaviruses (CoVs) infect birds and mammals. Human coronaviruses (HCoVs) 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, that caused severe acute respiratory syndrome (SARS) [11]. This virus was called SARS-CoV and it caused 8000 human infections and 774 deaths in 37 countries during 2002–2003 [12]. In 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV) emerged, which was first detected in Saudi Arabia [13], causing 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 belongs to the *Betacoronavirus* genus originating from bats. SARS-CoV-2 has ~79% sequence similarity to SARS-CoV and 50% to MERS-CoV [2]. SARS-CoV-2 is postulated to use the angiotensin-converting enzyme 2 (ACE2) receptor to infect human cells, based on its structural similarity to SARS-CoV in its receptor-binding domain [2,16]. Wang and Cheng 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 host cells, facilitating its infection and spread [17].

4. Structure of SARS-CoV-2

The structure of the SARS-CoV-2 virion is comprised of a spike (S) glycoprotein, a haemagglutinin-esterase (HE) dimer, a membrane (M) glycoprotein, an envelope (E) protein, the nucleocapsid (N) protein and the RNA genome [2]. The spike (S) glycoprotein is highly glycosylated and uses an *N*-terminal signal sequence to enter the endoplasmic reticulum and bind to receptors of the human host cell. The S glycoprotein determines the tissue tropism of the virus, that is the affinity of SARS-CoV-2 towards the host cell. SARS-CoV-2 binds to the ACE2 receptor expressed on pneumocytes [17,18]. Binding to the ACE2 receptor triggers conformational changes in the S glycoprotein, allowing its cleavage by the transmembrane protease TMPRSS2 and the release of S fragments into the cell supernatant, which inhibit virus neutralisation 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 and subsequently packaging the genome encapsulated in viral particles. The envelope (E) glycoprotein is probably a transmembrane protein with functions of acting as an ion channel, facilitating the assembly and release of the virus. The membrane (M) protein is present as a dimer in the virion and can have two different conformations allowing it to promote membrane curvature and joining the nucleocapsid. Finally, the hemagglutinin-esterase (HE) dimeric glycoprotein binds to sialic acids in surface glycoproteins [16].

5. Pharmacodynamics

Studies have shown that CQ and HCQ may have antiviral activity through various mechanisms (Fig. 1), as described below.

5.1. Prevention of virus entry into the cell

Many viruses invade cells 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].

5.2. Altered virus replication

Viruses use the host cell machinery to produce their progeny. Some enveloped viruses additionally require posttranslational modification 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 the possible effects of CQ/HCQ in 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 Flaviviridae family 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 human immunodeficiency virus type 1 (HIV-1) gp120 envelope glycoprotein and inhibits replication of HIV in $CD4^+$ T-cells, producing non-infectious retrovirus particles [34–37].

5.3. 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 mouse studies, CQ can prevent vertical transmission of Zika virus by the maternal-fetal pathway [39].

5.4. Immune modulation

The CQ/HCQ-induced pH elevation in cellular organelles may have the effect of inhibiting the production of various cytokines, chemokines or immune-modulating mediators, the 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 (SLE). The main mechanism of this immunomodulatory

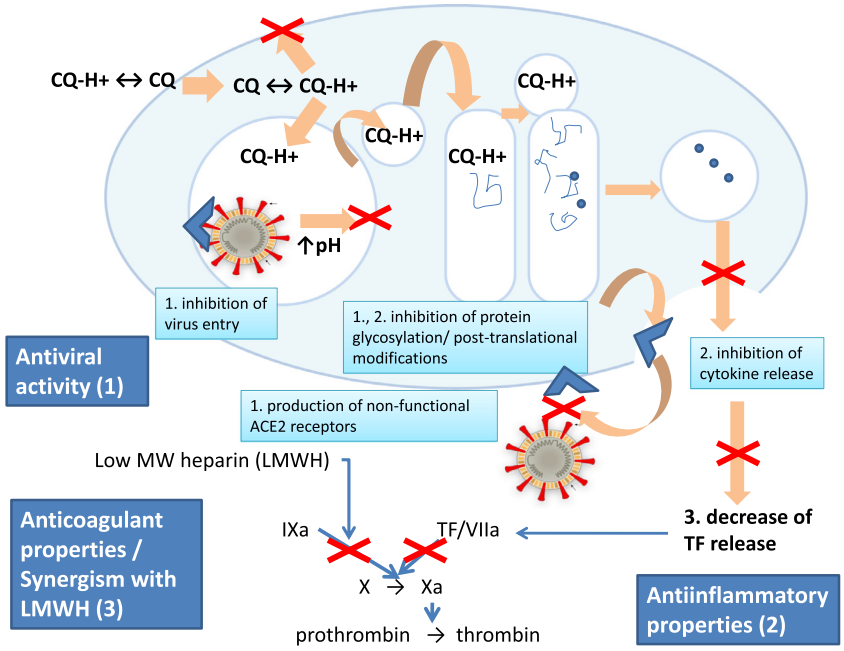

Fig. 1. Specific potential mechanisms of action of chloroquine (CQ) and hydroxychloroquine (HCQ) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). LMWH, low-molecular-weight heparin; ACE2, angiotensin-converting enzyme 2; TF. tissue factor.

action is partly mediated by a reduction in tumour necrosis factor (TNF) at the level of monocytes–macrophages [40–42].

5.5. Anticoagulant activity

An anticoagulant activity of aminoquinoline drugs has been reported since the 1960s [43]. CQ was reported to inhibit the alternative pathway of the complement system 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 higher than those likely to be achieved 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 by-product of its anti-inflammatory activity. This is in line with the anticoagulant effects of the drug reported in individuals with SLE [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 on factor X and impeding thrombus formation during COVID-19. This drug combination has become part of the standard of care in Italy [49].

5.6. 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: prevention of viral entry; impaired replication; and a pleiotropic action on the human immune system through its immunomodulating 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.

5.6.1. Possible chloroquine/hydroxychloroquine mechanism of action at the ACE2 receptor level

Previous studies in SARS-CoV discovered a binding affinity between the ACE2 receptor and the viral S glycoprotein [50]. The mechanism of action of CQ against SARS-CoV may be the induction of surface expression of subglycosylated ACE2, as the alteration of terminal glycosylation of ACE2 decreases the binding affinity between the human ACE2 receptor and the SARS-CoV S glycoprotein, thus preventing virus entry into 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 appears to be more easily transmitted [47]. Wang and Cheng 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].

5.6.2. Possible chloroquine/hydroxychloroquine 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 CQ 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].

5.6.3. 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 CQ, namely PLpro, which is one of the two viral cysteine proteases involved in posttranslational 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, namely the 3-chymotrypsin-like protease ($3CL^{pro}$), is the putative target for lopinavir, originally developed as an anti-HIV drug [56].

5.6.4. Immunomodulatory activity

In the immunopathogenesis of severe cases of SARS, a phenomenon that worsens the damage caused by the virus is called the '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, interferon-gamma (IFNγ)-inducible protein 10 (IP-10), interleukin-6 (IL-6) and IL-8 [58,59]. A similar phenomenon called 'cytokine storm' has been observed in patients with SARS-CoV-2 who display high levels of IL-1β, IFNγ, IP-10 and monocyte chemoattractant protein-1 (MCP-1), which probably lead to activated T-helper 1 (Th_1) cell responses. Patients with severe SARS-CoV-2 infection requiring admission to the intensive care unit (ICU) had higher concentrations of granulocyte colony-stimulating factor (G-CSF), IP-10, MCP-1, macrophage inflammatory protein 1-alpha (MIP-1α), MIP-1β and TNF-α than those who did not require ICU admission, suggesting that the 'cytokine storm' was associated with the severity of 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 (Th_2) cytokines (e.g. IL-4 and IL-10) that suppress inflammation, a phenomenon that differs from SARS-CoV infection. In the pathophysiology of this 'cytokine storm' associated with SARS-CoV-2, the ACE2 receptor appears to play an important role. The hypothesis that the ACE2 is a receptor 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 SARS-CoV-2 not only damages 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.

6. 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 at high doses for long periods with very good tolerability [53]. Following oral administration, CQ/HCQ are widely and slowly distributed throughout the body due to extensive sequestration in tissues, particularly the liver, spleen, kidney, lung, melanin-containing tissues and, to a lesser extent, the brain and spinal cord [62]. This large apparent volume of distribution confers to CQ/HCQ a relatively short plasma half-life. CQ/HCQ accumulate 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, with the main cell permeant being the unprotonated form of CQ that represents a minority of the extracellular CQ pool. According to the Henderson–Hasselbalch equation, however, part of the remaining CQ portion dissociates to maintain equilibrium at physiological pH, thus allowing the drug to gradually enter cells. As passage through the plasma membrane is due to diffusion and not active transport, 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 concentration more quickly. Once inside cells, CQ/HCQ is protonated at a rate inversely proportional to the pH, again according to the Henderson-Hasselbalch 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]. The drug is approximately 4–5-fold more concentrated in whole blood than in plasma owing to this intracellular accumulation [67]. For this reason, whole blood levels of the drug represent a more meaningful marker for its pharmacokinetics than plasma levels. Among the different cell types, the drug is largely accumulated in tissue macrophages that 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 hoarding of the aminoquinolines also represents a basis for their slow excretion. CQ/HCQ is maintained within the body for prolonged periods after its withdrawal. For example, HCQ has a half-life of 2963 h [68]. Clearance to the extracellular environment of CQ and HCQ is by exocytosis and/or through the action of the multidrug resistance protein 1 (MRP-1), a cell surface drug transporter belonging to the ATP-binding cassette (ABC) family, which also includes P-glycoprotein [37,69]. HCQ is metabolised in the liver into three active metabolites, namely desethylchloroquine (DCQ), desethylhydroxychloroquine and bisdesethylhydroxychloroquine [70].

DCQ possess anti-Zika virus activity [71]. All of the *N*-dealkylated metabolites have been implicated in heart failure and retinopathy due to long-term treatment with CQ [72]. CQ and DCQ concentrations decline slowly, with elimination half-lives of 20–60 days [73]. CQ clearance is by the renal route and 38% of the administered dose is eliminated without changes [74].

7. Use of chloroquine/hydroxychloroquine in SARS-CoV-2 infection

7.1. 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 HCoV-OC43 in newborn mice; 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 clinical use [76]. In a study with BHK-21 cells infected with recombinant human coronavirus rHCoVs-OC43 labelled with Renilla luciferase, CQ inhibited the replication of rHCoV-OC43 in vitro [77].

There are three in vitro studies on 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 [half maximal effective concentration (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 of HCQ sulfate orally twice daily,

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 (EC_{50} = 1.13 μM; 50% cytotoxic concentration (CC_{50}) > 100 μM; selectivity index (SI) > 88.50]. The 90% effective concentration (EC_{90}) 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 Vero E6 cells from green monkey kidney (ATCC 1586), finding that it efficiently inhibits SARS-CoV-2 infection [4]. In addition, the study confirmed that HCQ inhibits the entry of SARS-CoV-2 into cells as well as the stages following SARS-CoV-2 entry, and CQ had similar effects [4].

7.2. Human clinical studies

The results of a number of clinical trials and observational studies [80–99] have been reported so far, many of which present methodological limitations due to conditions of duress of an unexpected pandemic (Table 1). Two studies also suffer from poor reporting, with no dosage being declared [88,95] and one of them including in the HCQ arm patients with worse baseline characteristics than the control group [88].

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 both because of reported antiviral activity and inhibition of immune activation [100], showing the dose dependency of the positive outcomes.

Seven of the COVID-19 clinical studies [81,83,87,93,94,96,97] were conducted with a median dosage of 400 mg/day of HCQ, with or without a loading dose (LD) and combined or not with azithromycin (AZM). Two of these studies [including one randomised clinical trial (RCT)] resulted in positive outcomes, and five studies (again, including one RCT) report negative results. One of these studies, however, reported results comparing the use of HCQ with that of another antiviral agent, lopinavir/ritonavir (LPV/r) [94]. The two observational studies conducted with the same dosage preceded by a LD resulted in opposite results, with the trial using the higher LD (800 mg/day) reporting positive results [99]. 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) [86,91,92,98,101]. Some of the studies using 600 mg of HCQ daily combined HCQ with AZM apart from an observational study, which showed negative results [82,89,91].

One RCT of HCQ using a 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 filed [80], the authors showed that after stratification of the patients by background antiviral therapy, the use of HCQ decreased the risk of hospitalisation. The reason why the authors removed this analysis in the subsequent version of the study is unclear [80]. As of 17 May 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 negativisation [85,90]. Finally, one study [84] reports the results of a trial including two arms, one arm of which was 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 an increased number of deaths.

Another recent study merits being dealt with in particular detail because of the level of alarm raised through its large media coverage and the high number of people on whom it was conducted [101]. After conducting a retrospective analysis of 671 hospitals in six continents, Mehra et al. concluded that CQ and HCQ, particularly in combination with macrolide antibiotics, increased the number of deaths in hospitalised patients with COVID-19 and that this excess mortality was associated with increased arrhythmias. However, the study is biased by the non-homogeneous distribution of pre-existing risk factors. For example, the treatment groups had a higher incidence of current cigarette smoking and hypertension and a higher body mass index (BMI), all factors in general associated with poorer prognosis. 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 and non-CQ/HCQ groups is not reported. It is known that some antivirals such as LPV/r, when administered at full dosages, can increase the incidence of arrhythmias [102] and this analysis should therefore have been reported. Finally, the study failed to detect the contribution of cigarette smoking to the incidence of arrhythmias, an association that is largely documented in the literature [103]. Despite these limitations, the study supports the notion of cautious monitoring of patients receiving CQ/HCQ, 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. A study by Silva Borba et al. administering the highest CQ dosage, however, is biased by the fact that the authors administered such a high dosage of CQ concomitantly with AZM, for reasons that will be apparent below [84]. 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 Gonçalves et al. [104], recent pharmacokinetic analyses [105] and some immune-modulating properties of the drug [106], Savarino and Tarek 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 [107]. The results of this modelling study also highlight a problem underlying many of the aforementioned clinical studies, which were conducted in patients already hospitalised: an antiviral effect of HCQ is to be expected when the drug is administered immediately early after diagnosis, before patients are hospitalised.

Finally, in regard of very early administration, a recently published study showed a potential for HCQ as post-exposure prophylaxis [108]. The study reports on a post-exposure prophylaxis regimen that was conducted in 211 patients and healthcare workers following exposure to two infected healthcare workers. After a median period of 10 days of preventive treatment with HCQ (400 mg/day), no-one tested positive for the virus. Unfortunately, there was no control group. The results of a controlled clinical trial of HCQ prophylaxis will soon be available [109].

8. Adverse drug reactions (ADRs)

ADRs related to CQ/HCQ can be generally divided into two types depending on the duration of administration. The first type of ADR

Table 1
Studies on the effectiveness and safety of chloroquine (CQ) and hydroxychloroquine (HCQ) in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Reference	Institution/country study conducted in	Study design	No. of patients	Treatment regimen/ duration (days)	Results		Adverse drug reactions	Authors' conclusions
					Primary outcome	Secondary outcome(s)		
Tang et al. (2020) [80]	16 Chinese government-designated COVID-19 centres in 3 provinces (Hubei, Henan, Anhui), China	Open-label, RCT; ITT analysis	150	HCQ 1200 mg/day LD D1–D3, followed by 800 mg (D4 up to D14 for mild/moderate symptoms; D4 up to D21 for severe symptoms) + SOC (included use of antivirals)	Negative SARS-CoV-2 conversion probability by 28 days in SOC plus HCQ group (85.4%, 95% CI 73.8–93.8%) was similar to that in the SOC group (81.3%, 95% CI 71.2–89.6%) ($P > 0.05$). Between-group difference was 4.1% (95% CI −10.3% to 18.5%)	• Probability of symptom alleviation by 28 days was similar between patients with SOC with and without HCQ [59.9% (95% CI 45.0–75.3%) vs. 66.6% (95% CI 39.5–90.9%); $P > 0.05$]. • Median time to alleviation of clinical symptoms: SOC + HCQ group vs. SOC group (19 days vs. 21 days; HR = 1.01, 95% CI 0.59–1.74; $P = 0.97$ by log-rank test)	Diarrhoea, 10% Blurred vision, 1.4% (transient with a period of 1–2 days)	Administration of HCQ did not result in a significantly higher negative conversion probability than SOC alone in patients mainly hospitalised with persistent mild-to-moderate COVID-19. Adverse events were higher in HCQ recipients than in non-recipients
Chen Z. et al. (2020) [83]	RenMin Hospital of Wuhan University, Wuhan, China	Double-blind, RCT; ITT analysis	62	HCQ 400 mg D1–D5 + SOC	• Time to clinical recovery (TTCR), body temperature recovery time and cough remission time were significantly shortened in the HCQ treatment group. • Patients progressing to severe illness in control and HCQ groups: 4/31 (12.9%) vs. 2/31 (6.45%)	• Absorption of pneumonia on chest CT: control group 17/31 (54.8%) vs. HCQ group 25/31 (80.6%). • Fever resolution (mean ± S.D.): control group 3.2 ± 1.3 days vs. HCQ group 2.2 ± 0.4 days ($P = 0.0008$) Cough remission (mean ± S.D.), control group 3.1 ± 1.5 days vs. 2.0 ± 0.2 days ($P = 0.0016$)	• Control group (0%) vs. HCQ group (6.4%) (rash, headache)	Among patients with COVID-19, use of HCQ could significantly shorten the TTCR and promote the absorption of pneumonia
Chen et al. (2020) [81]	Shanghai Public Health Clinical Center Shanghai, China	Open-label, RCT; ITT analysis	30	HCQ 400 mg D1–D5 + SOC	On D7, COVID-19 nucleic acid of throat swabs was negative in 86.7% in the HCQ group and 93.3% in the control group ($P > 0.05$)	Radiological progression was shown on CT images in 5/15 cases (33.3%) in the HCQ group and 7/15 cases (46.7%) in the control group, and all patients showed improvement in follow-up examinations	Four cases (26.7%) in the HCQ group and 3 cases (20.0%) in the control group had transient diarrhoea and abnormal liver function ($P > 0.05$)	Prognosis of moderate COVID-19 patients is good. Larger sample size studies are needed to investigate the effects of HCQ in the treatment of COVID-19
Gautret et al. (2020) [82]	IHU Méditerranée Infection, Marseille, France	Open-label, non-randomised clinical trial; PP analysis	42	HCQ 600 mg D1–D10 ± AZM 500 mg LD, then 250 mg D2–D5 + SOC	At D6 post-inclusion, 70% of HCQ-treated patients were virologically cured vs. 12.5% in the control group ($P = 0.001$)	Drug effect was significantly higher in patients with symptoms of URTI and LRTI compared with asymptomatic patients ($P < 0.05$)	No data	Despite its small sample size, the survey shows that HCQ treatment is significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by AZM
Silva Borba et al. (2020) [84]	Hospital e Pronto-Socorro Delphina Rinaldi Abdel Aziz, Manaus, Western Brazilian Amazon	Double-blind, phase IIb RCT	440	High-dose CQ (600 mg CQ twice daily for 10 days or total dose 12 g) or low-dose CQ (450 mg for 5 days, twice daily only on D1, or total dose 2.7 g)	The high-dose arm presented more QTc > 500 ms (18.9%) and a trend toward higher lethality (39%) than the low-dose arm. The fatality rate until D13 was 27% (95% CI 17.9–38.2%), overlapping with the CI of historical data from similar patients not using CQ (95% CI 14.5–19.2%)	In 27 patients with paired samples, respiratory secretion at D4 was negative in only six (22%)	The high-dose CQ arm presented more QTc > 500 ms (18.9%) and a trend toward higher lethality (39%) than the low-dose CQ arm	Preliminary findings suggest that the higher CQ dosage (10-day regimen) should not be recommended for COVID-19 treatment because of its potential safety risks

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Reference	Institution/country study conducted in	Study design	No. of patients	Treatment regimen/ duration (days)	Results		Adverse drug reactions	Authors' conclusions
					Primary outcome	Secondary outcome(s)		
Huang et al. (2020) [85]	12 hospitals in Guangdong and Hubei Provinces. China	Multicentre prospective observational study	197	CQ 500 mg, orally, twice (half dose) or once (full dose) daily, D1–D10	Median time to achieve undetectable viral RNA was shorter with CQ than non-CQ therapy (absolute difference in medians, −6.0 days, 95% CI −6.0 to −4.0 days; $P < 0.0001$)	• Duration of fever was shorter in CQ (geometric mean ratio 0.6, 95% CI 0.5–0.8; $P = 0.0029$). • 1/197 patients (0.5%) in the CQ group experienced aggravated symptoms from moderate to severe, whilst 9/176 patients (5.1%) in the non-CQ group had the same aggravated experience	CQ vs. non-CQ group: - any adverse events, 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) [86]	Assistance Publique-Hôpitaux de Marseille (AP-HM), in IHU Méditerranée Infection, Marseille, Southern France	Observational study	1061	HCQ (200 mg three times daily for 10 days) + AZM (500 mg on D1 followed by 250 mg daily for the next 4 days) for ≥3 days	Good clinical outcome and virological cure obtained in 973 patients (91.7%) within 10 days	A poor clinical outcome was observed for 46 patients (4.3%) and 8 (0.8%) died (74–95 years old). All deaths resulted from respiratory failure and not cardiac toxicity	Mild adverse events, 2.3% (gastrointestinal or skin symptoms, headache, insomnia and transient blurred vision)	Administration of HCQ + AZM combination before COVID-19 complications occur is safe and is associated with very a low fatality rate
Yu B. et al (2020) [87]	Tongji Hospital, Wuhan, China	Observational study	568	HCQ 200 mg twice daily for 7–10 days	Mortality was 18.8% (9/48) in the HCQ group and 45.8% (238/520) in the non-HCQ group ($P < 0.001$)	Level of inflammatory cytokine IL-6 was significantly lowered from 22.2 (8.3–118.9) pg/mL at the beginning of treatment to 5.2 (3.0–23.4) pg/mL at the end of treatment in the HCQ group ($P < 0.05$) but there was no change in the non-HCQ group	No data	HCQ treatment is significantly associated with decreased mortality in critically ill patients with COVID-19 through attenuation of the inflammatory cytokine storm. Therefore, HCQ should be prescribed for treatment of critically ill COVID-19 patients to save lives
Mallat et al. (2020) [97]	Cleveland Clinic Abu Dhabi, UAE	Retrospective observational study	34	HCQ 400 mg twice daily for 1 day, followed by 400 mg daily for 10 days	Time to SARS-CoV-2 negativity was significantly longer in patients who received HCQ compared with those who did not [17 (13–21) days vs. 10 (4–13) days; $P = 0.023$]	No patients were admitted to the ICU, required high-flow oxygen therapy or non-invasive or invasive mechanical ventilation, and all of them were discharged alive from the hospital	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

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Reference	Institution/country study conducted in	Study design	No. of patients	Treatment regimen/ duration (days)	Results		Adverse drug reactions	Authors' conclusions
					Primary outcome	Secondary outcome(s)		
Magagnoli et al. (2020) [88]	Data from patients hospitalised with confirmed SARS-CoV-2 infection in all US Veterans Health Administration medical centres until 11 April 2020	Retrospective observational study	368	Exposure to HCQ alone or with AZM as treatment in addition to standard supportive management for COVID-19	Compared with the no-HCQ group, there was a higher risk of death from any cause in the HCQ group (adjusted HR = 2.61, 95% CI 1.10–6.17; $P = 0.03$) but not in the HCQ + AZM group (adjusted HR = 1.14, 95% CI 0.56–2.32; $P = 0.72$) • **Important**: baseline pulse oximetry (SpO_2) >95%: HCQ, HCQ+AZM and no HCQ, 62.9%, 57.5% and 73.4%, respectively. There was a higher percentage of patients with $SpO_2 > 95$ in those who did not receive HCQ or HCQ+AZM	No significant difference in risk of ventilation in either the HCQ group (adjusted HR = 1.43, 95% CI 0.53–3.79; $P = 0.48$) or the HCQ+AZM group (adjusted HR = 0.43, 95% CI 0.16–1.12; $P = 0.09$) compared with the no HCQ group	No data	No evidence that use of HCQ, with or without AZM, reduces the risk of mechanical ventilation in patients hospitalised with COVID-19
Molina et al. (2020) [89]	Saint Louis Hospital, Paris, France	Prospective, uncontrolled, single-arm study	11	600 mg/day of HCQ for 10 days + AZM 500 mg on D1 followed by 250 mg/day for next 4 days	Nasopharyngeal swabs in 8/10 patients were still positive for SARS-CoV-2 RNA at D5–6 after treatment initiation		No data	No evidence of strong antiviral activity or clinical benefit of the combination of HCQ + AZM in severely ill COVID-19 patients
Gao et al. (2020) [90]	10 hospitals in China in cities of Wuhan, Jingzhou, Guangzhou, Beijing, Shanghai, Chingqing and Ningbo	Observational study	100	CQ 500 mg twice daily D1–D10 + SOC	100 patients demonstrated that CQ phosphate is superior to control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus-negative conversion and shortening the disease course according to a news briefing		Severe adverse reactions to CQ 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. (2020) [91]	IHU Méditerranée Infection, Marseille, France	Observational study	80	HCQ 600 mg D1–D10 + AZM 500 mg LD, then 250 mg D2–D5	Nasopharyngeal viral load 83% negative at D7 and 93% at D8; virus culture negativity from respiratory samples 97.5% at D5		Nausea or vomiting, 2.5% Diarrhoea, 5% Blurred vision, 1.2%	HCQ + AZM is effective in the treatment of COVID-19
Mahévas et al. (2020) [92]	French hospitals in adults with documented SARS-CoV-2 pneumonia requiring oxygen ≥ 2 L/min	Observational study	181	HCQ 600 mg/day	20.2% of 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; RR = 0.91, 95% CI 0.47–1.80)	2.8% of patients in the HCQ group died within 7 days vs. 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 (3–9) days, 9.5%	HCQ did not significantly reduce ICU admission or death at D7 after hospital admission, or ARDS in hospitalised patients with hypoxaemic pneumonia due to COVID-19

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Reference	Institution/country study conducted in	Study design	No. of patients	Treatment regimen/ duration (days)	Results		Adverse drug reactions	Authors' conclusions
					Primary outcome	Secondary outcome(s)		
Rosenberg et al. (2020) [93]	Inpatients admitted to hospitals in the New York City (NYC) metropolitan region between 15–28 March 2020, USA	Observational study	1438	HCQ 200–600 mg/day; dose and duration were variable	There were no significant differences in mortality for patients receiving HCQ + AZM (HR = 1.35 95% CI 0.76–2.40), HCQ alone (HR = 1.08 95% CI 0.63–1.85) or AZM alone (HR = 0.56, 95% CI 0.26–1.21)		No significant differences in relative likelihood of abnormal ECG findings. Diarrhoea: HCQ + AZM group (11.6%) vs. HCQ alone (17%) Hypoglycaemia: HCQ + AZM group (3.4%) vs. HCQ alone (0.5%). QT prolongation: HCQ + AZM group (11.6%) vs. HCQ alone (14.4%)	Among patients hospitalised in metropolitan NYC with COVID-19, treatment with HCQ, AMZ or both, compared with neither treatment, was not significantly associated with differences in in-hospital mortality
Geleris et al. (2020) [96]	New York–Presbyterian Hospital–Columbia University Irving Medical Center, USA	Observational study	1446	HCQ 600 mg twice on D1, then 400 mg daily for a median of 5 days	Primary endpoint was time from study baseline to intubation or death. For patients who died after intubation, the timing of the primary endpoint was defined as the time of intubation. There was no significant association between HCQ use and intubation or death (HR = 1.04, 95% CI 0.82–1.32)		No data	HCQ administration was not associated with either a greatly lowered or an increased risk of the composite endpoint of intubation or death
Shabrawishi et al. (2020) [95]	Tertiary public hospital in Mecca, Kingdom of Saudi Arabia	Observational study	93	CQ or HCQ with or without AZM. There were three interventional subgroups: group A ($n = 45$) who received antimalarial drug only (A1), combined with AZM (A2) or combined with antiviral drugs (A3); and group B, a supportive care group ($n = 48$)	Primary and secondary endpoints of the study were achieving negative SARS-CoV-2 nasopharyngeal PCR sample within ≤5 days from the start of the intervention and ≤12 days from the diagnosis, respectively. In group A, 73.3% (33/45) achieved the primary endpoint and 84.4% (38/45) achieved the secondary endpoint. Smaller percentages of 68.8% (33/48) and 79.2% (38/48) 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 or to accelerate the negative PCR conversion rate

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Reference	Institution/country study conducted in	Study design	No. of patients	Treatment regimen/ duration (days)	Results		Adverse drug reactions	Authors' conclusions
					Primary outcome	Secondary outcome(s)		
Lee et al (2020) [94]	Hospitals in Busan, South Korea	Observational study	72	HCQ 400 mg orally every 24 h for 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. Switching therapy due to clinical failure was significantly more common in the HCQ group than in the LPV/r group [40.7% (11/27) and 2.2% (1/45), respectively; $P = 0.001$]	Disease progression was also significantly more common in the HCQ group than in the LPV/r group [44.4% (12/27) and 17.8% (8/45), respectively; $P = 0.030$]	Experienced adverse effects: LPV/r (22; 49%) vs. HCQ (7; 26%). Drug interruption due to adverse effects: LPV/r (2; 4%) vs. HCQ (1; 4%)	LPV/r appears to be more effective than HCQ at preventing progression to severe disease in patients with COVID-19
Mehra et al (2020) [101] [a]	The registry comprised data from 671 hospitals in six continents, including patients hospitalised between 20 December 2019 and 14 April 2020 with a positive laboratory finding for SARS-CoV-2	Observational study	96 032	Mean (S.D.) daily dose and duration of various drug regimens were as follows: CQ alone, 765 (308) mg and 6.6 (2.4) days; HCQ alone, 596 (126) mg and 4.2 (1.9) days; CQ with a macrolide, 790 (320) mg and 6.8 (2.5) days; and HCQ with a macrolide, 597 (128) mg and 4.3 (2.0) days	After controlling for multiple confounding factors, compared with mortality in the control group (9.3%), HQC (18.0%; HR = 1.335, 95% CI 1.223–1•457), HQC with a macrolide (23.8%; HR = 1.447, 95% CI 1.368–1.531), CQ (16.4%; HR = 1.365, 95% CI 1.218–1.531) and CQ with a macrolide (22.2%; HR = 1.368, 95% CI 1.273–1.469) were each independently associated with an increased risk of in-hospital mortality	Compared with the control group (0.3%), HCQ (6.1%; HR = 2.369, 95% CI 1.935–2.900), HCQ with a macrolide (8.1%; HR = 5.106, 95% CI 4.106–5.983), CQ (4.3%; HR = 3.561, 95% CI 2.760–4.596) and CQ with a macrolide (6.5%; HR = 4.011, 95% CI 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, are associated with decreased in-hospital survival and an increased frequency of ventricular arrhythmias when used for treatment of COVID-19
Ahmad et al. (2020) [98]	Residents of three LTCFs in New York, USA	Observational study	54	Doxycycline (100 mg orally twice daily for 7 days) and HCQ (two regimens): (i) 200 mg orally three times daily for 7 days; or (ii) 400 mg orally twice daily on D1, then 400 mg daily for 6 days	85% of patients showed clinical recovery defined as: resolution of fever and shortness of breath, or a return to baseline setting if patients were ventilator-dependent	11% of patients were transferred to acute-care hospitals owing to clinical deterioration and 6% died. Naive indirect comparison suggests these data were significantly better outcomes than the data reported from a LTCF in King County, Washington where 57% of patients were hospitalised and 22% died [151]	2% had a seizure and HCQ was immediately terminated	Doxycycline + HCQ treatment in high-risk COVID-19 patients is associated with a reduction in clinical recovery, decreased transfer to hospital and decreased mortality
Membrillo de Novales et al. (2020) [99]	Inpatients from Central Defense Hospital 'Gómez Ulla', Madrid, Spain	Observational study	166	LD 800 mg + 400 mg, followed by a maintenance dose of 400 mg/day	• 48.8% of patients not treated with HCQ died vs. 22% of those treated with HCQ ($P = 0.002$). • According to clinical picture at admission, HCQ increased the mean cumulative survival 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, HCQ treatment with 800 mg added LD increased survival when patients were admitted in early stages of the disease

COVID-19, coronavirus disease 2019; RCT, randomised clinical trial; ITT, intention-to-treat; LD. loading dose; D, day; SOC, standard of care; CI, confidence interval; HR, hazard ratio; CT, computed tomography; S.D., standard deviation; PP, per-protocol; AZM, azithromycin; URTI, upper tract respiratory infection, LRTI, lower tract respiratory infection; IL, interleukin; ICU, intensive care unit; RR, relative risk; ECG, electrocardiogram; ARDS, acute respiratory distress syndrome; LPV/r, lopinavir/ritonavir; LTCF, long-term care facilities.

[a] This article has been retracted.

occurs when the drug is 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 a long period of time (years), as occurs in the treatment of SLE and rheumatoid arthritis, and caused by accumulation of the drug in the body ('cumulative toxicity') [110]. 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 [111]. Severe but very rare ADRs have been observed when administered for several years and occur due to the accumulation of drug in the body.

8.1. 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 that reported during administration in the treatment of malaria. For SARS-CoV-2 treatment, a duration of 5–20 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. For the treatment of malaria, the dose is 25 mg/kg for 3 days (in a 60 kg patient, 1500 mg/day) [112]. The most frequent CQ/HCQ-associated 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%) [113–117]. The most serious but very rare ADRs have been reported with treatment for >5 years, among which the two most important are 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 [111]. Reported cases of severe arrhythmias (torsades de pointes) or sudden death have been reported in patients receiving >5 years of treatment for autoimmune diseases [118].

Safety concerns have been raised for cardiac toxicity also during acute treatment with HCQ [118]. In this regard, important insights on safety issues can be derived from a recent survey on data from almost one million patients with autoimmune diseases treated with HCQ [119]. The results show that there is no risk of significant prolongation of the QT interval in patients treated with HCQ alone for <30 days in comparison with those treated with sulfasalazine. On the other hand, the risk was increased when HCQ was used in combination with AZM.

It may be argued that because COVID-19 causes cardiac problems, the cardiac toxicity of HCQ can be enhanced in the short-term. These considerations can be rejected in light of the fact that autoimmune diseases such as SLE and rheumatoid arthritis for which HCQ has been used for decades can also affect the heart. Moreover, a number of guidelines that have been issued to prevent and circumvent HCQ-related cardiac toxicity in patients with COVID-19 [118,120,121] would be highly recommended at this stage.

It has also been hypothesised that a short HCQ treatment might be detrimental in the treatment of COVID-19 because the drug may impair innate immunity and thus deprive the host from an important self-defence weapon against the virus [122]. These considerations, however, are only theoretical and appear 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 SLE showed that in fact the drug decreases infectious events [123]. Second, the HCQ analogue CQ was shown to significantly increase cell-mediated responses in response to a viral antigen [106,124]. Cell-mediated responses have recently been shown to play a major part in protection against SARS-CoV-2 in vivo [125].

8.2. Safety issues with chronic treatment

In a systematic study on chronic use (3.25–7.9 years) of CQ/HCQ in patients with SLE, HCQ had fewer adverse reactions than CQ. The proportions of ADRs were as follows: nausea (7–12%); diarrhoea (18%); myopathy (1.3%); headache (1.3–12%); ototoxicity (0.6%); and dermatological reactions such as urticaria (0.6–12%) [117]. The frequency of cardiotoxicity such as conduction disorders (0–4%) and cardiomyopathy (0–1.3%) was very rare [126]. The frequency of retinal toxicity ranged from 0.33–16%, and a study compared the frequency of retinal toxicity between CQ and HCQ reported 19% vs. 0%, respectively [127].

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 [128]. Chatre et al. conducted a systematic study on cardiotoxicity associated with CQ/HCQ; of the total 127 cases, 15% were patients on short-term treatment (malaria) and the remaining were patients on prolonged treatments for connective tissue diseases [110]. They found that cardiotoxicity was predominant in women (65%); the mean duration of use of CQ/HCQ was 7 years (range, 3 days to 35 years), higher in CQ users than HCQ, and with a high cumulative dose (median 1235 g for HCQ and 803 g for CQ). The most common CQ/HCQ-induced cardiac disorder was conduction disorders (85%), among which in order of frequency are atrioventricular block, first- and second-degree block, complete atrioventricular 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 patients (61%) 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 emphasise that this systematic study reviewed cases of cardiotoxicity for more than 40 years of CQ/HCQ use in the world (the study covers reports from 1975–2017) [110]. Acute cardiotoxicity occurs due to alteration in ion channels with a destabilising 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 vacuolisation of myocytes [110].

Keratoplasty and retinopathy induced by CQ/HCQ have not been described when used as an antimalarial. The frequency is very low and they have been described in patients who used HCQ for >10 years and at a high dose [129]. The incidence of HQC-induced retinopathy is 0.4% in patients whose daily dosage is >6.5 mg/kg or who have taken HCQ continuously for >10 years [130–132]. Bilateral pigmentary retinopathy induced by CQ/HCQ begins with subtle paracentral scotomas, followed later by 'bull's eye' maculopathy, which is characterised by a ring of retinal pigment epithelium (RPE) in the macular area closest to the fovea and the final stage with generalised RPE and atrophic retina with loss of central, peripheral and night vision. Risk factors for retinopathy are doses of CQ > 2.3 mg/kg and HCQ > 5.0 mg/kg, duration of therapy >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 examinations [133,134].

8.3. Precautions in the use of chloroquine/hydroxychloroquine 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, especially in the most severe

forms of presentation, precautions must be taken, which are listed in Supplementary Table S1. The Liverpool Drug Interaction Group (based at the University of Liverpool, UK), in collaboration with the University Hospital of Basel (Switzerland) and Radboud UMC (Nijmegen, the 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/).

9. Clinical practice guidelines in anti-SARS-CoV-2 antiviral therapy including chloroquine/hydroxy chloroquine

Currently, there are a number of on-line accessible clinical practice guidelines based on expert consensus in countries such as Belgium, the USA (2), China (3), Ireland, Italy (2), Pakistan, France, Spain, Ecuador and Iran. 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 5–10 days. In severe cases, administration of HCQ is suggested by nasogastric tube. On the 5th day, adverse reactions should be evaluated considering the long half-life [135]. In Hangzhou, China, the Zhejiang University School of Medicine suggested administering CQ in COVID-19-positive patients only if the basic regimen is not effective (LPV/r combined with Arbidol) [136]. The Multicenter Collaboration Group of the Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for chloroquine in the treatment of novel coronavirus pneumonia has indicated CQ in pneumonia in COVID-19-positive patients aged >18 years and <65 years [137]. The consensus suggests administering CQ phosphate, 500 mg each administration, twice daily for 10 days. If severe gastrointestinal reactions occur, the dose may be reduced to 500 mg/day or even discontinued. During the treatment course, if the test for throat swab coronavirus content becomes negative for 3 days, withdrawal of the drug may be considered, but the minimum course of treatment is 5 days. Precautions during treatment with CQ include monitoring full blood count, cardiac enzymes every 2 days, electrocardiogram before and after starting the drug (Days 5 and 10) and evolution of the clinical picture by chest computed tomography (CT) imaging [76,137]. In Ireland, the Health Service Executive (HSE) National Clinical Advisor and Group Lead, Acute Hospitals, suggest administration of CQ or HCQ to all confirmed patients with COVID-19 infection [138]. In Italy, the National Institute for the Infectious Diseases 'L. Spallanzani', IRCCS, suggested administration of HCQ associated with base therapy (e.g. LPV/r) in all confirmed patients with symptomatic COVID-19, lasting 10 days [139]. The Italian Society of Infectious and Tropical Diseases (SIMIT), Section of Regione Lombardia, suggested administering CQ or HCQ to all patients confirmed with COVID-19 over the age of 70 years and/or with risk factors and/or symptomatic. The duration of treatment can be from 5–20 days according to the severity of pneumonia. In severe cases, it suggested administering HCQ by nasogastric tube [140]. The COVID-19 management guidelines of the Pakistan Chest Society suggested administering a HCQ loading dose of 400 mg twice daily followed by 200 mg three times daily for 10 days, or CQ 500 mg twice daily for 10 days [141]. In the USA, the University of Washington School of Medicine suggested administering HCQ in cases confirmed with COVID-19, with risk factors and aged >60 years, with a duration depending on the severity of the case, from 5–10 days [142]. In France, SRLF-SFAR-SFMU-GFRUP-SPILF, Misson COREB Nationale, CQ is recommended at 500 mg twice daily. Alternatively, HCQ is recommended at 200 mg three times daily [143]. This dosage is higher than that recommended in other clinical guidelines, such as the Italian ones; 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, based on studies in antimalarial treatment. In Spain, the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) recommended a dose in adults of HCQ 400 mg twice daily on Day 1 followed by 200 mg twice daily for the rest of the course (5 days). Alternatively, CQ is recommended at 620 mg followed by 310 mg 12 h later on Day 1 followed by 310 mg twice daily for the rest of the course (5 days) [144]. In Ecuador, the Ministry of Public Health, the *Therapeutic Guide for COVID-19* indicates CQ/HCQ in hospitalised patients (ICU or ward) [145]. The Iranian Expert's Consensus Statement, the *Algorithmic Approach to Diagnosis and Treatment of Coronavirus Disease 2019 (COVID-19) in Children*, suggests the use of CQ, associated with other antivirals, in patients who are admitted to the ICU, combining antiviral agents and immunomodulators [146].

The Italian Medicines Agency (AIFA), having first authorised CQ/HCQ treatment also for non-hospitalised COVID-19 patients [147], has ceased recommending the use of CQ/HCQ for treatment of COVID-19 [148] following the aforementioned (now retracted) report of Mehra et al. [101]. Following the same report [101], France has also stopped recommending the use of CQ/HCQ [149]. The Spanish drug regulatory agency has instead decided to maintain the recommendation for HCQ treatment owing to the limitations of the aforementioned report [150].

10. Conclusion

In the current context of the COVID-19 pandemic, with disastrous health and economic consequences, it is important to consider all 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 actions 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 favourable risk/benefit balance, CQ/HCQ could become part of the pharmacological armamentarium in the war against SARS-CoV-2.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.ijantimicag.2020.106078.

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