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Review Article

The controversial therapeutic journey of chloroquine and hydroxychloroquine in the battle against SARS-CoV-2: A comprehensive review

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ABSTRACT

Recently, the pandemic outbreak of a novel coronavirus, officially termed as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), indicated by a pulmonary infection in humans, has become one of the most significant challenges for public health. In the current fight against coronavirus disease-2019, the medical and health authorities across the world focused on quick diagnosis and isolation of patients; meanwhile, researchers worldwide are exploring the possibility of developing vaccines and novel therapeutic options to combat this deadly disease. Recently, based on various small clinical observations, uncontrolled case studies and previously reported antiviral activity against SARS-CoV-1 chloroquine (CQ) and hydroxychloroquine (HCQ) have attracted exceptional consideration as possible therapeutic agents against SARS-CoV-2. However, there are reports on little to no effect of CQ or HCQ against SARS-CoV-2, and many reports have raised concerns about their cardiac toxicity. Here, in this review, we examine the chemistry, molecular mechanism, and pharmacology, including the current scenario and future prospects of CQ or HCQ in the treatment of SARS-CoV-2.

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1. Introduction

As of February 9, 2021, the number of confirmed coronavirus cases has risen to 107,107,663 worldwide, and 2,339,203 deaths are reported as a result of this deadly virus also known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It has spread terribly worldwide (Figure 1) [1]. Coronavirus disease-2019 (COVID-19) accounted for a public health crisis of national and international concern. Currently, there is no prominent, specific, highly effective, proven treatment available. Therefore, based on in vitro studies, it is proposed that chloroquine (CQ), which was conventionally used to treat malaria, has some beneficial effects in diminishing viral replication. CQ is also found to be effective in other viral infections such as SARS and Middle East respiratory syndrome coronavirus (MERS-CoV) and has been used worldwide for more than 70 years, and it is covered under the model list of essential medicines of the World Health Organization (WHO). Already in 2007, few authentic journals have shown that CQ may fight against orphan viral infections [2].

CQ and hydroxychloroquine (HCQ) are derivatives of quinine. HCQ is the amino anisotropic form, and the end of the CQ side chain (N-ethyl end) is bearing a hydroxyl group. For the prophylaxis of malaria, they are in prominent medications for decades and are used to treat autoimmune diseases, for instance, rheumatoid arthritis and systemic lupus erythematosus. They can stimulate the body's immune system through the inhibition of TLR7 and TLR9 and thereby enhance the production of cytokines, which could be useful to fight against this deadly disease. They also have a very efficient oral absorption profile making its administration easier. Moreover, they have high tissue sequestration due to the high volume of distribution.

As a robust bioactive drug, CQ/HCQ appears to possess antiviral activity against RNA viruses [3]. According to former studies, both these drugs show a broad spectrum of antiviral activity on various viruses like the Marburg virus, Ebola virus [4], Dengue virus [5], human immunodeficiency virus (HIV) [6], Zika virus [7], SARS-CoV-1 [8], Rabies virus [9], Poliovirus [10], Hepatitis A and C viruses, influenza A and B virus [11], and Chikungunya virus [12].

2. SARS-CoV-2 and some significant facts

WHO and other research organizations ought to repurpose the existing drugs whose safety profile is already accepted and recognized as an effective treatment for other diseases. Researchers are also looking forward to those molecules that have achieved good results in animal research against SARS and MERS. The main focus was on those candidates who are already engaged in successful activities for various viral diseases and malaria. Based on some in vitro and in vivo studies on MERS and SARS in 2017, CQ and HCQ were administered intravenously to the COVID-19 patients in the United States and EUROPE by reviewing their history. Figure 2 Explains the strategies that interfere with the replication of SARS-CoV-2 [13–19].

CQ and HCQ have attracted considerable attention as it diminishes the acidity in endosomes, a compartment that cells usually use to ingest outside material, which some viruses adopt during infection. Nevertheless, the pathway of entry of SARS CoV-2 is different from another; it usually uses their spike proteins to get attached to the receptor on the human cell surface. Studies show that CQ can destroy the virus, but it requires a comparatively high dose, which cannot be considered beneficial. Moreover, WHO commented, “no data has been shared” for more than 20 patients in China treated with this drug. As the whole world is still under the grip of this pandemic, researchers are continuously trying to develop the best possible treatment to combat this deadly disease. Some of them put their views on the combination of Lopinavir-Ritonavir. This combination has an effective property to inhibit the HIV-1 protease (an enzyme that cleaves a long-chain protein during the assembly of new viruses) [1,20–22].

3. Mechanism of action of CQ/HCQ on viral diseases

The interference of CQ with the viral particle attaches to their cell surface receptor and inhibits the viral cycle at its pre-entry step. CQ inhibits

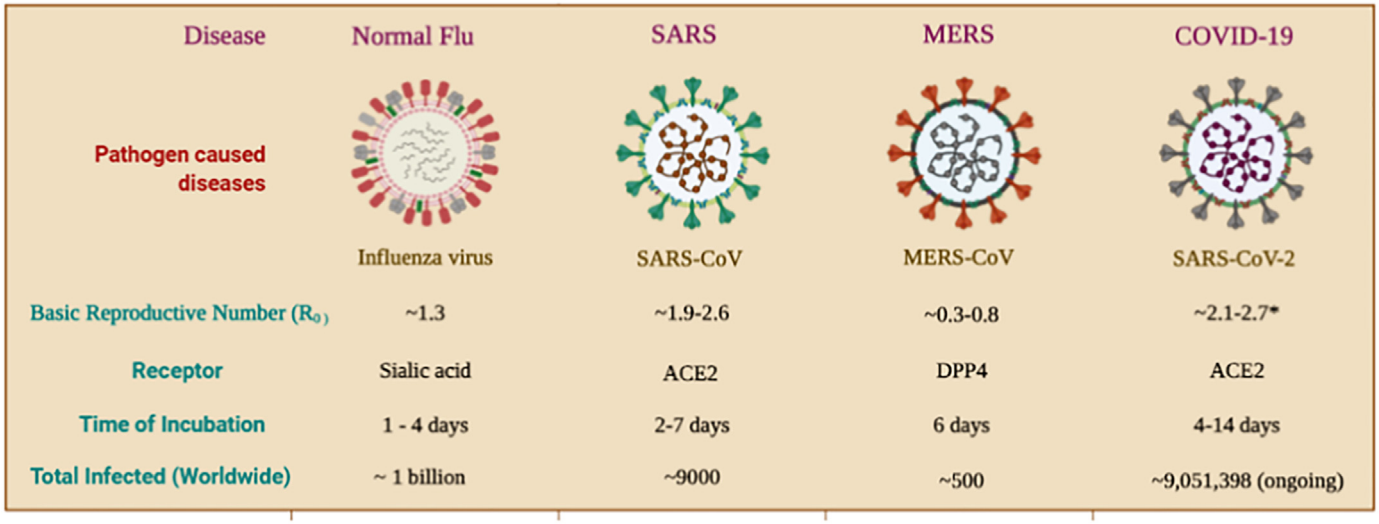

Figure 1. Comparison of the different respiratory viral infections epidemiology. *Current ongoing data of COVID-19 shown in the diagram.

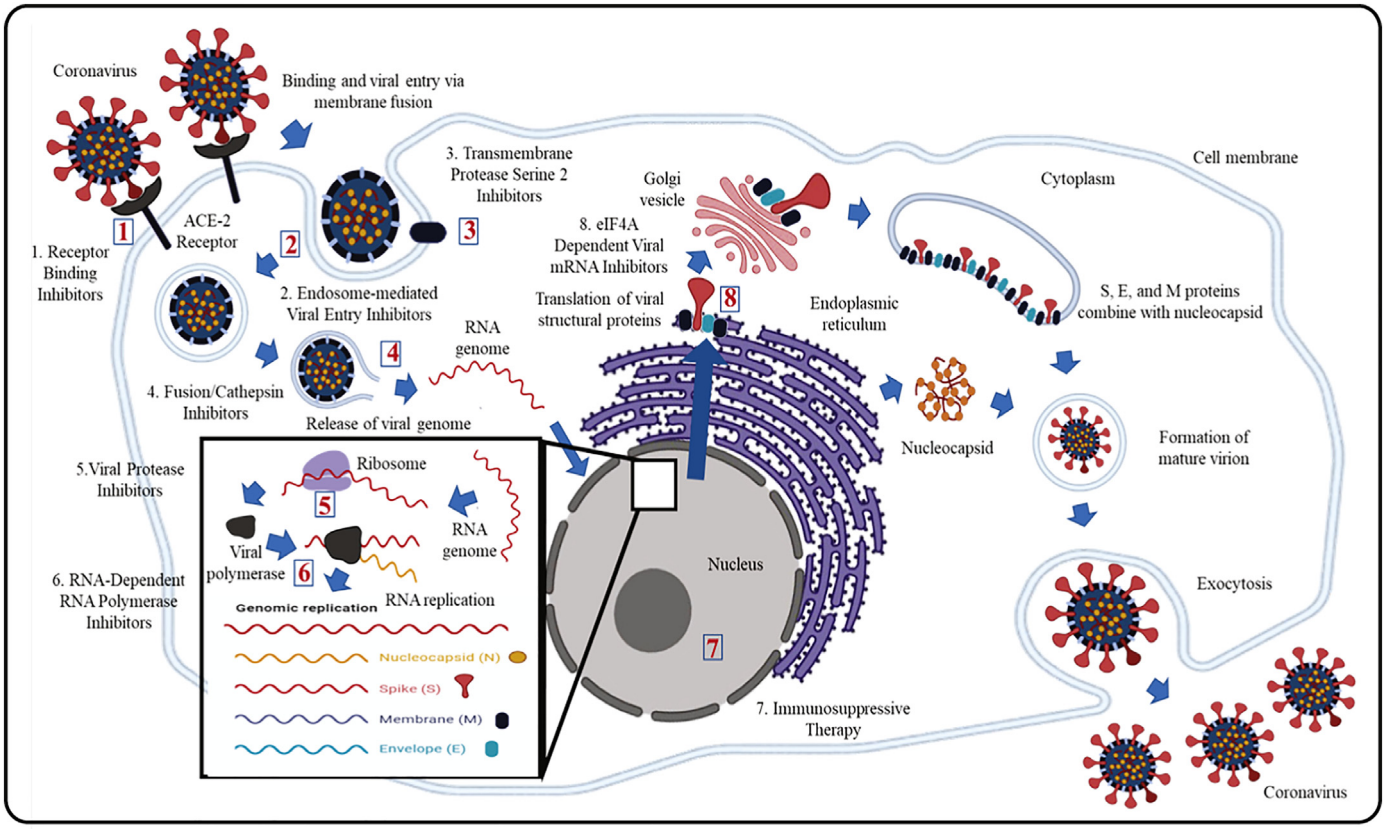

Figure 2. Pictorial representation of strategies to interfere with the steps of the replication cycle of SARS-CoV-2 [13–19].

quinone reductase 2 (structurally related to UDP-N acetylglucosamine 2-epimerases) associated with sialic acid biosynthesis [22]. The sialic acids are the acidic monosaccharides, which are essential components of ligand recognition and are seen at the end of sugar chains that exist on cell transmembrane protein. As viruses such as orthomyxoviruses and human coronaviruses usually use such sialic moieties as their receptors, CQ can be accounted for the broad-spectrum antiviral activity as it can interfere with sialic acid biosynthesis [23]. It also impairs the replication of virus at its early stage by the interruption of the pH-dependent endosome-mediated viral access of enveloped viruses like Chikungunya virus or Dengue virus. CQ was found to be useful in the in vitro treatment of the Chikungunya virus because of the alkalization of endosomes after addition to Vero cells preceding viral exposure [10]. The virus is inhibited fundamentally by the blockage of endocytosis and a quick raise in endosomal pH and abrogate virus-endosome fusion. According to reports, SARS-CoV-1, after the attachment of the DC-SIGN receptor, activates endosomes at acidic pH as the mechanism behind the entry of the virus into the target cells is completely pH-dependent, which results in a fusion of endosomal membranes and the virus leads to the detachment of viral SARS-CoV-1 genome into the intracellular fluid.

CQ can also block the entire replication cycle, which can eventually inhibit the hepatitis A virus associated with uncoating. CQ's antiretroviral effect is depicted by the inhibition of glycosylation of the gp120 envelope glycoprotein posttranscriptionally, as it can be stated that CQ interferes with the posttranslational refinement of viral proteins, which include proteases and glycosyltransferases present in the endoplasmic reticulum or the trans-Golgi network vesicles, which may need a lower pH, and the viral infection is impaired as the neosynthesized virus fragments are not infectious [24]. In the study using a nonhuman coronavirus, it is found that the intracellular location of coronavirus sprouting is controlled by the localization of its membrane M proteins, which gathered in the Golgi complex outside the spot of virion sprouting [25]. Therefore, a few suggestions were received on the probable activity of CQ on SARS-CoV-2 at this stage of the viral replication cycle. According to recent reports, the C-terminal end of the MERS-CoV M protein consists of a trans-Golgi network localization signal. CQ has a profound activity inhibition of phosphorylation of the p38 mitogen-activated protein kinase (MAPK) in caspase-1 and THP-1 cells. Thus, it can also control the immune system through cell signaling and regulate pro-inflammatory cytokines. Cell stimulation through MAPK signaling is essential more often to attain their reproduction cycle [26]. Inhibition of p38 MAPK occurs in the model of HCoV-229 coronavirus by using CQ [27]. According to reports, CQ also suppresses interleukin-1 beta (IL-1β) mRNA expression in THP-1 cells and decreases IL-1 β release. A CQ-induced drop of IL-1 and IL-6 cytokines in monocytes/macrophages [28] and the inhibition of tumor necrosis factor-alpha (TNFα) production by immune cells were studied through the interruption of cellular iron metabolism. Similarly, CQ also causes the inhibition of TNFα mRNA expression and inhibition of the conversion of pro-TNF into soluble mature TNFα molecules. The inhibition of the TNFα receptor by CQ was also studied in U937 monocytic cells [29].

4. Medicinal chemistry point of view of CQ/HCQ

CQ or 4-N-(7-chloroquinolin-4-yl)-1-N,1-N-diethylpentane-1,4-diamine is a quinoline ring-based antimalarial drug with a molecular weight of 319.9 g/mol and HCQ or 2-[4-[(7-chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol is a derivative of CQ with additional hydroxyl group substituted on terminal N-ethyl group with a molecular weight of 335.9 g/mol (Fig. 3) [30]. Illustrates the chemical structure of CQ and HCQ [24,25].

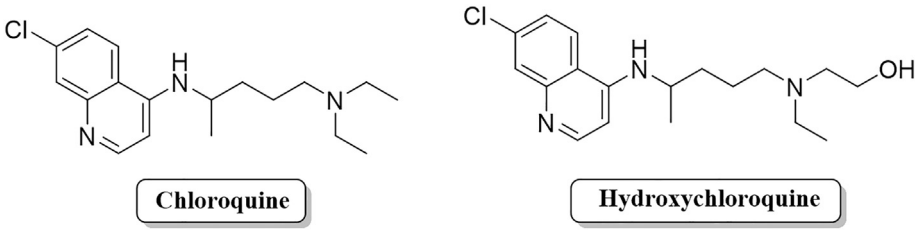

Figure 3. Chemical structure of CQ and HCQ.

5. Common pharmacokinetics profile of CQ/HCQ

Both CQ and HCQ have the same quinolone parent ring system and are derivatives of 4-aminoquinoline (4AQ) that share similar pharmacology with slight changes in the details [31].

5.1. Absorption

After the oral administration of CQ and HCQ, they absorb completely within 2 to 4 h with slight variation among the subjects [32,33]. The mean absorption of both drugs is almost equal [34] and normally gets absorbed in the upper part of the intestinal tract [35,36]. CQ overdose could be treated when the subject is administered with charcoal orally by reducing absorption [37].

5.2. Distribution

Both have a large distribution volume, and they sequestrate to various tissues like the kidney, liver, spleen, and lung tissues. Also, it can bind extensively to melanin-containing tissues. Both of them bind to albumin and alpha1-acid glycoprotein with different binding capacity [38]. For HCQ, (*S*)-enantiomer shows a better plasma protein binding than (*R*)-enantiomer [39]. CQ binds to corneal melanin more strongly than HCQ. Therefore, HCQ is associated with a lower risk of retinopathy [40,41].

5.3. Metabolism

Metabolism of CQ and HCQ is done in the liver for dealkylation by cytochrome *p*450 [42]. The number of metabolites formed after the metabolism of both CQ and HCQ are different. CQ has two metabolites (desethylchloroquine and bisdesethylchloroquine), and HCQ has three metabolites (desethylchloroquine, desethylhydroxychloroquine, and bisdesethylhydroxychloroquine) [43]. These metabolites that are formed also have pharmacological activities like the parent drug. In all, 30–79% of the oral drug is metabolized, and the remaining is excreted. Even after a single dose of CQ, the drug and its metabolites might present in urine for months [44].

5.4. Excretion

CQ and HCQ are mainly metabolized by the kidney and liver, and the metabolized or unchanged drug is excreted through urine or feces [45]. The skin is the other organ through which a small amount of drug is excreted. People with kidney or liver dysfunction are at a higher risk of retinopathy [46,47]. The alkalinity of urine decreases the excretion of CQ [48].

6. Drug–drug interaction

CYP450 is the enzyme responsible for the dealkylation of CQ and HCQ. This is the enzyme involved in the metabolism of many other drugs, and therefore, there is a chance for interaction with other drugs [49,50]. The enzymes, namely CYP3A4, CYP2C8, CYP1A1, and CYP2D6, can metabolize CQ [51].

The use of digitoxin and CQ increases the level of digitoxin in plasma [52]. HCQ prevents the metabolism of metoprolol as it competes with CYP2D6, and there will be an increased plasma concentration of metoprolol. In the same way as plasma, the dextromethorphan concentration is also increased with the concurrent use of HCQ [53]. Tamoxifen, a selective estrogen receptor modulator used in breast cancer treatment, increases the retinopathy when used with either CQ or HCQ. It synergistically inhibits lysosomal enzymes in the retina [54]. HCQ and methotrexate, when administered together, may reduce the absorption of methotrexate through a change in pH [55]. The dose of cyclosporine should be monitored as cyclosporine levels can be increased along with HCQ [56]. Drugs such as proton pump inhibitors may alter the bioavailability and absorption of CQ and HCQ by changing the gastric pH [57]. Both CQ and HCQ can cross the placenta, but toxicity to the fetus has not been reported [58,59], and a small amount of the drug is excreted into breast milk [60]. Drug–drug interaction of CQ/HCQ with other COVID-19 treatment drug data is still missing, which has also raised a question on the usage of these drugs, necessitating the need for future studies in this area.

7. CQ/HCQ toxicity profile

Both CQ and HCQ show a good safety profile. They can trigger immune responses through TLR7 and TLR9 signaling inhibition, and this could be made use of in SARS CoV-2. Treatment with CQ or HCQ does not risk infections or cancer as in other immunosuppressant drugs [61]. The most common toxicity of these drugs is associated with gastrointestinal toxicities like nausea, vomiting, abdominal discomfort, and diarrhea [62]. Another toxicity that is reported by the use of CQ and HCQ is an occurrence of myopathy and arrhythmia, and prolonged QT interval. Usage for a longer period, like in rheumatic patients, may cause myopathy [63]. Development of retinopathy is the most complicated toxicity of the drugs reported so far, and it is more common with CQ than HCQ. CQ may cause lysosomal degradation of photoreceptor and hence retinal damage [64].

8. *In silico* studies of CQ/HCQ against SARS-CoV-2

Viral nucleoprotein, a complex nucleocapsid protein (N) and positive-sense RNA, is essential to replicate the virus. N protein has two terminals: N terminal and C terminal. The main viral protease has an important role to cause infection in the host body. The receptor-binding domain (RBD), a part of the viral spike protein, facilitates the virus to attach to the ACE2 receptor [65]. An inhibitor that acts on the viral main protein would work as a solution for this viral infection, *in silico* screening or molecular docking studies of the existing antiviral drugs, including CQ and HCQ, around the world [65,66]. These studies mainly focus on binding affinities of drugs of interest to four main targets such as viral main protease (M^{pro}), host cathepsin L (CTSL), ACE2, and RBD of viral spike protein. In a study reported by Braz et al. (2020), CQ and HCQ were docked using Autodock Vina® on viral main protease (PDB ID: 6LU7) X-ray crystalline structure

Table 1
Binding affinities of CQ and HCQ with SARS-CoV-2 viral proteins

Sl. no	Entry	Binding affinities (kcal/mol)			
		M^{pro}	CTSL	ACE2	RBD
1.	CQ	−7.9	−5.4	−4.2	−4.2
2.	HCQ	−6.5	−5.2	−8.5	−6.5

of M^{pro} with an inhibitor N3 having a resolution of 2.16 A. Binding affinities of CQ and HCQ are given in the following table [67]. Table 1 reveals the binding affinities of CQ and HCQ with SARS-CoV-2 viral proteins.

An *in silico* study by Srivastava et al. [68] on the main protease of virus reports that CQ and HCQ show binding with GLY143 (Bond length 2.321 Å) and PHE140 (Bond length 2.501 Å), respectively, with a binding affinity of −8.15 and −7.62 Kcal/mol. They further find log P and Log S, which is 5.18 and −4.26 for CQ, whereas for HCQ, it is 3.87 and −4.11, respectively. To investigate the association of CQ and HCQ with the NTD-N-protein of SARS-CoV-2, Amin et al. conducted an *in silico* study [69] to explore the possible role of both drugs for the treatment of SAR-CoV-2 infection using different computational approaches by comparing the ability of both drugs on its binding to NTD-N-protein. HCQ demonstrated interesting binding energy of −7.28 kcal/mol against NTD-N-protein when compared with CQ binding energy of −6.30 kcal/mol. CQ shows interactions with VAL156, LEU159, GLN160, LEU161, LEU167, and ALA173. On the other hand, HCQ shows interaction with VAL72, ILE74, THR135, PRO162, and GLY69. A study using CQ for potential *in silico* interactions against both RBD-ACE-2 and NTD-ganglioside, Fantini et al. [65] have helped identify CQ to interfere with the initial binding of virus particles to the respiratory tract surface epithelium. This study evidence supports the usage of CQ as an initial treatment for SARS-CoV-2-infected patients. Sachdeva et al. [70] in a study revealed that CQ and HCQ shows the glide score of −4.02 (PDB id: 6M0J), −3.97 (PDB id: 6YLA) and −6.23 (PDB id: 6M0J), and −4.74 (PDB id: 6YLA) against the RBD of spike protein and −4.23 and −5.63 (PDB id: 6LU7), respectively, against RBD of main protease (M^{pro}). They also further demonstrate some crucial bindings. Nimgampalle et al. [71] performed molecular docking and associated studies between CQ and its derivatives and SARS-CoV-2 virus proteins. The results reveal that both CQ and HCQ can attach to unique structural and nonstructural proteins involved in SARS CoV-2 infection pathogenesis with diverse efficacies. It also consists of several chemically synthesized CQ derivatives that can prevent various SARS-CoV-2 virus proteins by tethering to them and proficiently interrupting these proteins' active sites simultaneously. Kalaria et al. [72] conduct an *in silico* study that reveals some important binding information of HCQ with the different proteins of SARS-CoV-2.

In another study, they attempted to dock CQ and HCQ to the RNA-binding domain of the virus's nucleocapsid phosphoprotein (NTD-N-protein), which is a capsid-like structure inside that the genetic matter of virus is present and N protein. NTD-N-protein helps the virus to invade the human cell and hence replication. They selected NTD-N-protein (PDB id: 6VYO) and used Autodock for the docking studies. Interaction of CQ and HCQ with the viral protein showed good binding affinities. While preparing the ligands, many conformers were generated, and the binding affinities ranged from −6.30 kcal/mol to −5.6 kcal/mol for CQ and −7.10 kcal/mol to −4.24 kcal/mol for HCQ. They also displayed different kinds of interactions [69]. From these studies, it is evident that CQ and HCQ can interact with different viral proteins. Hence, they appear to be promising molecules for the detailed research in the treatment of SARS-CoV-2 infection.

9. Pharmacological treatment with promising clinical benefits

Concerning coronaviruses, the promising pharmacological advantages of CQ were significantly described for SARS-CoV-1 long back. In 2006, Biot and colleagues already conducted a relative inhibitory activity study of CQ and HCQ against SARS-CoV-1 in Vero cells. This study established that CQ had around fivefold augmented potency (EC_{50} of 6.5 ± 3.2 μM) in comparison to HCQ (EC_{50} of 34 ± 5 μM) [73]. In early February, Wang and colleagues illustrated strong in vitro activity of CQ in COVID-19 with an EC_{50} of 1.13 μM in Vero E6 cells after 48 h [21]. The information was consistent with the previous reports of CQ's inhibitory activity against SARS-CoV-1 and MERS-CoV in different cell lines. EC_{50} values ranged from 1 to 8.8 μM for SARS-CoV-1 and 3.0 μM for MERS-CoV were manifested [74]. To date, China conducted 15 clinical trials to study the safety and efficacy of CQ or HCQ in COVID-19 treatment; 8 were for CQ, 6 were for HCQ, and the remaining involved both of them [75]. Thus far, the CQ phosphate group shows an effective increase in the negative rate of virus nucleic acid test, reduction in the worsening of pneumonia, and improvement of lung imaging findings in a clinical trial of above 100 patients. Keeping these findings in mind, the Guidelines (version 6) for the treatment of COVID-19 suggests CQ phosphate be administered by an oral dose of 500 mg (300 mg for CQ) for adults, twice a day (not more than 10 days). "HCQ's therapeutic effect on new coronavirus (COVID-19)" was registered (NO: ChiCTR2000029559) [76]. As of February 17, 20 patients were entitled to the basic treatment group and HCQ group. After 1–2 days of medication, clinical indications were improved in all of them, and after 5 days, an improvement was observed in the lung imaging reports on 19 patients. Additionally, no patients had a worsening condition of illness in the HCQ group. On adjusting the dosage regimen, the adverse reactions (like slight headache and mild rashes) that occurred because of drug intake disappeared.

CQ was also notably observed to suppress in vitro replication of HCoV-229E in cultures of epithelial lung cells. In a study in 2009, it was found that fatal infections of newborn mice with the HCoV-O43 coronavirus could be prevented by treating it with CQ through mother's milk. In vitro tests also give evidence of a solid antiviral effect of CQ on recombinant HCoV-O43 coronavirus. According to an in vitro study, CQ was documented as an active drug against MERS-CoV though this thought remains controversial [77]. Despite all these, China and France's primary experiences are inspiring the world because of the promising role of CQ, or instead HCQ, in the management of COVID-19. In a Chinese study, around 100 patients infected with SARS-CoV-2 were treated with CQ and experienced fast relief from fever and improved lung CT [78]. These people took a shorter duration for cure as compared to the control group. Apart from its minor risks like retinopathy (on cumulative dose) [79] and rarely reported cardiac myopathy [80], CQ was earlier considered as the best available treatment for the virus infection as any other specific drugs were not invented for the same [3].

Numerous studies have revealed the efficacy of CQ/HCQ against coronaviruses, including the SARS-associated coronavirus, for reasons that are possibly partially similar involving phagolysosome CQ alkalization [81–83]. The in vitro activity of CQ against SARS-CoV-2 was discovered in China using culture tests on Vero E6 cells at 50% and 90% effective concentrations (EC_{50} and EC_{90} values) of 1.13 μM and 6.90 μM, respectively [84]. There were recent studies conducted to prove the antiviral effect of CQ and its derivatives on different cell lines. Researchers used Vero E6 cells obtained from African green monkey, BALB/c mice, Crandell-Reese feline kidney cells (CRFK), human epithelial lung cells (L132), HRT-18 cells, Huh7 cells, and *Felis catus* fetus-4 cells to prove the antiviral effect [85].

In a study conducted by Keyaerts et al. [82], the antiviral EC_{50} on the SARS-CoV-1 virus for CQ was found to be 8.8 ± 1.2 μM. They have performed their antiviral screening on Vero E6 cells from the African green monkey's kidney. Vincent et al. [86] researched the antiviral activity of CQ using Vero E6 cells and reported an EC_{50} value of 4.4 ± 1.0 μM. In another study, Barnard et al. [8] proved the antiviral activity of CQ, CQ monophosphate, and CQ diphosphate with an EC_{50} of 1–4 μM, 4–6 μM, and 3–4 μM, respectively. They used both Vero76 cells and BALB/c mice. Antiviral activity of CQ and HCQ with an EC_{50} of 6.5 ± 3.2 μM and 34 ± 5 μM on Vero E6 cells on SARS-CoV was reported by Biot et al. [32]. In the same work, they have reported the activity of both CQ and HCQ on cat coronavirus using CRFK cell lines, and the EC_{50} was >0.8 μM and 28 ± 27 μM, respectively. Kono et al. [27] reported CQ for HCoV-229E on L132 with an activity of 10 μM and 25 μM, respectively. The HCoV-OC43 virus was targeted in another study by Keyaerts et al. [87], and the activity for CQ was proved on HRT-18 cells with EC_{50}, 0.306 ± 0.0091 μM. *Feline catus* whole fetus was used to prove the action of CQ on feline infectious peritonitis virus (FIPV), and the inhibition was in a dose-dependent manner [88]. In another study reported by De Wilde et al. [76], CQ was reported against SARS-CoV, MERS-CoV, and HCoV-229E-GFP (recombinant) with EC_{50} of 4.1 ± 1.0 μM, 3.0 ± 1.1 μM, and 3.3 ± 1.2 μM. They have used

the human liver cell line (Huh7) and Vero E6 for the work. Again, Vero E6 cell lines are used to prove the activity of CQ on SARS-CoV-2 with EC_{50} of 1.13 μM [84].

A study by Gao et al. [89] found that CQ could minimize the duration of hospital stay and improvement in the development of COVID-19 pneumonia, which leads to the recommendation that patients with mild, moderate, and extreme types of COVID-19 pneumonia should be given 500 mg of CQ twice a day. A therapeutic concentration of CQ could be achieved at such a dose. Colson et al. [85], in a study, stated that as the mode of action of these two molecules is similar, the effect of HCQ on viruses is likely to be the same as that of CQ, which would therefore be the only option for COVID-19 treatment. A loading dose followed by a maintenance dose should be provided for optimal treatment. The optimum dose of CQ/HCQ for SARS-CoV-2 was an issue faced by the doctor during therapy.

A study conducted by Guanguan Li et al. concluded that the enantiomers of CQ and HCQ act differently against SARS-CoV-2, and S enantiomers were found to be more effective. S enantiomers showed a better hERG inhibition and M^{pro} activity in vitro as well as S-HCQ in vivo QT prolongation than R enantiomers [90].

10. Current scenario and future perspectives

Timely treatment of COVID-19 infection increases the chance to recover fast and helps to avoid its serious effects. Drug repurposing is the most effective and fastest way to identify a molecule that can combat the virus. These molecules have already passed all the hurdles of drug discovery processes. Comorbidities like cardiovascular diseases are to be considered because among the people affected by COVID-19, a huge number of them are elderly people, having other underlying diseases like cardiac and lifestyle disorders like diabetes [20,91–93]. General supporting treatments like electrolyte maintenance in the body and maintaining all vitals like heart rate, pulse rate, and respiratory rate is essential. Clinical trials are being conducted worldwide on different drugs. Antivirals such as interferon-alpha [94] (inhibit virus replication and induce innate and adaptive immunity), Lopinavir/Ritonavir [95] (inhibiting virus replication by interfering with protease enzyme), ribavirin [96] (nucleoside analog inhibits RNA and DNA virus replication), CQ and HCQ [5] (broad-spectrum antiviral used in malaria and autoimmune diseases), arbidol [63] (anti-influenza drug by inhibiting the reproduction of virus), and Remdesivir [97] (nucleoside analog) might be promising in the treatment of COVID-19. Many of these drugs are used globally by choice, as no specific drugs are available. Cellular therapy with natural killer cells and mesenchymal stem cells are other choices to enhance the body's immune response [98,99]. Antiviral antibodies extracted from recovered humans can also be utilized as passive immunization because this type of plasma therapy was effective in the case of Ebola, influenza, and poliomyelitis [44]. Monoclonal antibodies can be used to neutralize the virus as they bind with spike protein and prevent the virus to enter the host [100]. In different countries, various clinical trials on vaccines are currently going on in different phases, including the Russian vaccine (Sputnik V) and another developed by AstraZeneca and the University of Oxford researchers [101–103]. The long-term effects of the virus infestation are unknown. It may affect organs other than the lungs like the liver, kidney, GI organs, and CNS. It is crucial to design and develop drugs or vaccines against this global threat and to repurpose studies for approved drugs. The various molecular mechanisms of CQ by which it can attain such outcomes are still necessary to explore further. As SARS-CoV-2 has been found to use the same ACE2 receptor as SARS-CoV-1, it can also be concluded that CQ interacts with ACE2 receptor glycosylation, which also helps prevent SARS-CoV-2 from binding to target cells [104,105]. Wang et al. [104] suggested that SARS-CoV and MERS-CoV are upregulated in the expression of ACE2 in the lung tissue, a mechanism that could speed their replication and spread. CQ therapy will impact this interaction if SARS-CoV-2 targets the sialic acid on certain cell subtypes like other coronaviruses [106,107]. Simmons et al. [108] concluded in a preliminary study that CQ interferes with SARS-CoV-2 in an attempt to acidify lysosomes and presumably inhibits cathepsins that need a low pH

Table 2
Clinical trials CQ, HCQ, and in combination with other drugs for COVID-19 treatment

Sl no.	Compound name	Clinical trial phase	Administration route	Sponsor name	ClinicalTrials.gov. identifier
1.	CQ	Phase 2 Phase 3	Oral	HaEmek Medical Center, Israel	NCT04333628
2.		Phase 4	Oral	Wroclaw Medical University	NCT04331600
3.		Phase 2	Oral	Oxford University Clinical Research Unit, Vietnam	NCT04328493
4.		Phase 2	Oral	Oxford University Clinical Research Unit, Vietnam	NCT04328493
5.		Phase 3	Oral	Washington University School of Medicine	NCT04333732
6.	HCQ	Phase 3	Oral	Dr. Michael Hill	NCT04329611
7.		Phase 3	Oral	University Hospital Tuebingen	NCT04340544
8.		Phase 2	Oral	Ravi Amaravadi, MD	NCT04329923
9.		Phase 2	Oral	Baylor Research Institute	NCT04333225
10.		Phase 2	Oral	ProgenaBiome	NCT04335084
11.		Early Phase 1	Oral	Rambam Health Care Campus	NCT04323631
12.		Phase 3	Oral	Barcelona Institute for Global Health	NCT04331834
13.		Phase 2	Oral	Columbia University	NCT04318444
14.		–	Oral	Services Institute of Medical Sciences, Pakistan	NCT04370015
15.		Phase 3	Oral	Louisiana State University Health Sciences Center in New Orleans	NCT04363450
16.		Phase 3	Oral	Shanghai Public Health Clinical Center	NCT04261517
17.	Dexamethasone and HCQ	Phase 3	Oral	Centre Chirurgical Marie Lannelongue	NCT04347980
18.	HCQ and Azithromycin	Phase 2	1. HCQ (oral) 2. Azithromycin (IV)	University of New Mexico	NCT04458948
19.		Phase 2	Oral	Duke University	NCT04335552
20.		Early Phase 1	Oral	Azidus Brazil	NCT04348474
21.		Phase 2	Oral	National Institute of Allergy and Infectious Diseases (NIAID)	NCT04358068
22.	CQ or HCQ	–	Oral	University of Oxford	NCT04303507
23.	HCQ and Lopinavir/Ritonavir	Phase 3	Oral	University of Melbourne	NCT04483960
24.		Phase 2	Oral	Asan Medical Center	NCT04307693
25.	Favipiravir HCQ Lopinavir/Ritonavir	–	Oral	Baqiyatallah Medical Sciences University	NCT04376814
26.	HCQ Ivermectin	Phase 3	Oral	Centenario Hospital Miguel Hidalgo	NCT04391127
27.	HCQ Sulfate Ascorbic Acid	Phase 2 Phase 3	Oral	University of Washington	NCT04328961
28.	Favipiravir combined with HCQ	Phase 3	Oral	Ministry of Health, Turkey	NCT04411433
29.	HCQ and Nitazoxanide	Phase 2 Phase 3	Oral	Tanta University	NCT04361318

for optimal cleavage of SARS-CoV-2 spike protein, a prerequisite for autophagosome formation. [84]. By reducing the production of proinflammatory cytokines and activating CD8-positive T cells, this drug could directly function in COVID-19 disease. But there was also a need for more research to fully prove this fact. Earlier reports have demonstrated that CQ is favored in treating by reducing the worsening of pneumonia, rising lung-imaging tests, promising virus-negative transformation, and decreasing the period of illness. CQ is a low-priced and promising drug, and has been in use for 70 years, which has proved to be a potential drug in the treatment of COVID-19 according to the clinical trial report (Table 2) [1]. HCQ is a comparatively safe drug used for curing many old-time disorders. Toxic consequences could occur when HCQ is administered in high doses or for a prolonged duration of treatment. No other safe alternatives appear to be highly successful in this time of misfortune. In vitro studies suggested that SARS-CoV-2 was restricted to human cells by inhibiting coronavirus-targeted cell receptor glycosylation and increasing endosomal pH, thereby decreasing endosome-mediated viral entry, which led to the use of HCQ as a possible therapy for COVID-19. Besides, HCQ decreases the generation of many proinflammatory cytokines in developing acute respiratory distress syndrome, a serious manifestation of COVID-19. These causes, coupled with widespread availability, oral administration, and presumed protection based on historical use in the treatment of malaria and other diseases, have resulted in widespread clinical use in COVID-19. The FDA released an Emergency Usage Authorization for HCQ to care for adults hospitalized with COVID-19 on March 28, 2020, which was subsequently withdrawn on June 15, 2020. The findings of a clinical trial conducted on hospitalized patients with COVID-19 for 14 days show that HCQ was not effective in the treatment of COVID-19, which is consistent with the results of recent in vitro studies indicating no clinical benefit from the antiviral activity of HCQ against SARS-CoV-2 and open-label pragmatic studies in the United Kingdom and Brazil [109].

Solidarity is an international clinical trial launched by the WHO and collaborators to help identify an appropriate cure for COVID-19. It is among the biggest global randomized trials for the treatment of COVID-19, which enrolled approximately 12,000 patients in over 30 countries at 500 hospital sites. The Solidarity Trial assesses the impact of drugs on 3 significant outcomes in patients with COVID-19: mortality, need for ventilation assistance, and hospital stay length. To determine their relative efficacy against COVID-19, the Solidarity Trial measures treatment choices to the standard of care. The Solidarity Trial aims to determine whether either medication enhances survival or reduces the need for ventilation or hospital stay length by the enrolment of patients in several countries. On July 4, 2020, the WHO approved the decision of the International Steering Committee (ISC) of the Solidarity Trial to discontinue the HCQ and lopinavir/ritonavir weapons of the trial. ISC made the above recommendation presented at the WHO COVID-19 Science and Innovation Summit on 1–2 July, based on the various therapies arising from the Solidarity Trial. Various other studies concluded HCQ and lopinavir/ritonavir having little to no effect on COVID-19 hospitalized patients than the standard treatment. Solidarity Trial investigators interrupted the trials with immediate effect. On October 15, 2020, the Solidarity Trial released the interim findings. All four tested therapies (remdesivir, HCQ, lopinavir/ritonavir, and interferon) were found to have little or no effect on overall mortality, ventilation onset, and hospital stay period in hospitalized patients. To continue the quest for successful COVID-19 therapeutics, the Solidarity Trial considers evaluating other therapies [110].

11. Conclusion

COVID-19 exhibits fever, tiredness, and dry cough as signs of the infection. The primary prerequisite for therapy is diverse treatments, including antiviral medicines. Promising ability to suppress in vitro replication of several coronaviruses was shown by CQ and HCQ. Even though the use of CQ and HCQ remains a questionable topic for the scientific community, based on their ability to suppress in vitro replication of several coronaviruses, it is still used to treat COVID-19 in some countries due to the lack of other adequate medication. The hypothesis that CQ/HCQ could enhance patients' clinical outcomes with SARS-CoV-2 is confirmed. Few reports indicate that it has some activity against the viral infection. On the other hand, some reports also highlight that it has nothing to do with the virus infection, which is still debatable. However, the safety and efficacy of CQ/HCQ for the treatment of COVID-19 are still not completely clear. Molecular mechanisms underlying CQ/HCQ effectiveness, however, remain to be further explored. The chemistry, pharmacology, including pharmacokinetics followed by the molecular mechanism behind CQ/HCQ toward SARS-CoV-2 is covered in this review with their current scenario with future prospects for COVID-19 treatment.

CRediT author statement

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Conflict of interest

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