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Possible mechanistic insights into iron homeostasis role of the action of 4-aminoquinolines (chloroquine/hydroxychloroquine) on COVID-19 (SARS-CoV-2) infection

G.E.-S. BATIHA[1], H.M. SHAHEEN[1], H.M. AL-KURAISHY[2], J.O. TEIBO[3], O.A. AKINFE[4], A.I. AL-GARBEE[2], T.K.A. TEIBO[5], S.M. KABRAH[6]

[1]Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, AlBeheira, Egypt
[2]Department of Clinical Pharmacology and Therapeutic Medicine, College of Medicine, Almustansiriyiah University, Baghdad, Iraq
[3]Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil
[4]Department of Biochemistry, University of Ibadan, Ibadan, Nigeria
[5]Department of Maternal-Infant and Public Health Nursing, College of Nursing, Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[6]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al-Qura University, Kingdom of Saudi Arabia

Abstract. – OBJECTIVE: With the recent direction in drug repurposing, many approved drugs have been evaluated to assess their effect on the coronavirus or SARS-CoV-2 infection (COVID-19). Driving this path, chloroquine (CQ) has been used in the treatment of malaria and hydroxychloroquine (HCQ) in immunomodulatory and anti-thrombotic action, playing a leading role in initial management of the viral infection.

MATERIALS AND METHODS: Literature search was done using Google Scholar, PubMed and Scopus database using keywords "chloroquine" "SARS-CoV-2" "COVID-19" "mechanism of action" and articles of interest were selected providing evidence of the possible role of CQ in viral infection.

RESULTS: In a bid to understand how and if CQ and HCQ would exert their anti-viral property, mechanistic exegesis was done to review various proposed mechanisms of action. This revealed the inhibition of viral attachment and entry, inhibition of enveloped glycoprotein, inhibition of the development and proliferation of new viral particles as the way they perform their action. There is an interplay between iron metabolism and homeostasis with COVID-19 infection and viral reproduction.

CONCLUSIONS: This study aims to show the functional role of CQ and HCQ, as well as to provide possible mechanistic insight on the role of iron on viral infection, iron starvation and its downstream cellular pathways involving hepcidin and proinflammatory cytokines. The overall aim of providing possible mode of action of CQ and HCQ in the management of COVID-19 infection is exhibited via its anti-viral, anti-inflammatory and anti-thrombotic activities.

Key Words:
COVID-19, Chloroquine/Hydroxychloroquine, Iron homeostasis, Mechanism of action, SARS-CoV-2.

Introduction

In the treatment of malaria in the last few decades, chloroquine (CQ) and various analogs such as hydroxychloroquine (HCQ) and 4-aminoquinolones have played frontline roles (Figure 1). However, this has been replaced with newer antimalarial drugs due to drug-resistant strains of *Plasmodium falciparum.* The immunomodulatory properties as well as anti-thrombotic properties particularly HCQ have been utilized in the treatment of anti-phospholipid syndrome (APS), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). It has been clearly shown that CQ and HCQ are good in the management of viral infections because they might potentially inhibit viral entry and spread from various *in vitro* and *in vivo studies*. The application of these drugs has been proposed on several human viruses, espe-

Corresponding Authors: Gaber El-Saber Batiha, MD; e-mail: gaberbatiha@gmail.com
John Oluwafemi Teibo, MD; e-mail: johnteibo@usp.br

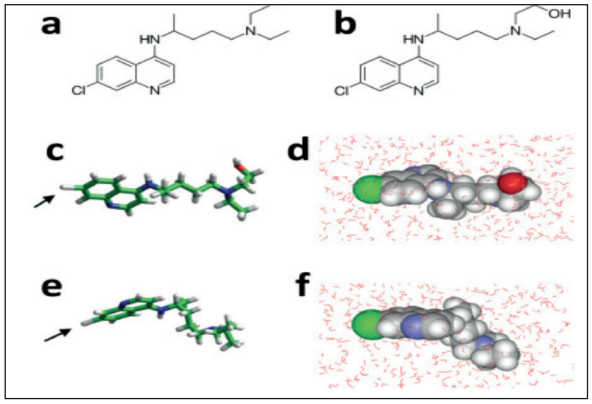

Figure 1. Chloroquine (CLQ) and hydroxychloroquine (CLQ-OH) chemical structures. **(a)** CLQ. **(b)** CLQ-OH. **(c)** CLQ-OH expanded conformer. **(d)** Aqueous CLQ-OH. **(e)** Typical dense conformer of CLQ. **(f)** Aqueous CLQ. The molecules in **(c–f)** appear in cylindrical or Spherical renderings (carbon, green; nitrogen, blue; oxygen, red; hydrogen, white). In **(c)** and **(e)**, the chlorine atom of CLQ and CLQ-OH is shown using an arrow[3].

cially Coronavirus like SARS-CoV and SARS-CoV-2. At the moment, fact-based findings on their application in human viral infections need elucidation[1].

Translation science from the bench to bedside should be established from comprehensive results, observational results as well as randomized experiments from scientific archives that have looked into the drug potency, duration of use and toxicity. These could be valuable in the prediction of their true efficacy. CQ sulfate and phosphate salts have both been endorsed as medications against malaria. HCQ has equally been utilized as a medication for malaria and auto-immune diseases, like lupus and rheumatoid arthritis. It is noted that CQ or HCQ are relatively safe with moderate and transitory side effects. In any case, there is a thin line between the treatment and toxic dose; the toxicity associated with CQ can lead to life-threatening cardiovascular disorders[2].

Effect of Chloroquine on Cellular and Biochemical Indices

Weak bases like CQ or HCQ influences acid vesicles by causing loss of enzymes function. Extracellularly, chloroquine or hydroxychloroquine have protonated structures, hence its positive charge and its inability to move across the plasma layer. Notwithstanding, the intracellular compartment permits the entry of the non-protonated part, hence the pH is inversely proportional to its protonation, just like Henderson-Hasselbach law. Therefore, chloroquine or hydroxychloroquine is abundant in the acidic organelles, for example, CQ or HCQ atoms are positively charged mainly because the Golgi vesicles, lysosomes, and endosome have low pH[4]. CQ or HCQ is expelled to the extracellular space generally by exocytosis and multidrug resistance protein MRP-1 activity, through a cell surface drug carrier ATP-binding cassette family and P-glycoprotein[5-7]. It has been proven that weak bases restrain post-translational modifications of nascent proteins by interrupting many enzymes such as acid hydrolases through the elevation of lysosomal pH and trans-Golgi network (TGN) vesicles. The rise in endosomal pH regulates iron metabolism inside the cells, reduces ferrated transferrin endosomal iron release and intracellular iron concentration all through the action of CQ. This reduction, influences the capacity of some enzymes activating cellular DNA replication and the gene expression pathways[8,9].

Antiviral Activity of the Drug Chloroquine or Hydrochloroquine

Antiviral activity is one of the proposed mechanism for both CQ and/ or HCQ. The previous research explains this activity by pointing out their weak basic nature. HCQ is an analogue of CQ, in the golgi it brings about increased acidic pH in the intracellular organelles, autophagy in vesicles, endosomes, lysosomes, bringing about autophagic flux[10] of the virus. CQ or HCQ could inhibit viral multiplication, replication and entry into various systems[11,12-15] even in SARS-CoV-2 disease. As far back as 1960, CQ antiviral action has been proven[16] to cause delay in the progression of a wide range of viruses in cell culture[17]. The evidence of its activity in mice has been found in many viral infections, including human COVID OC43[18], enterovirus EV-A71[19], Zika infection[20] and flu A H5N1[21].

Endosome-Mediated Viral Entry Interaction

A number of virus use endocytosis as a mechanism to permeate their target cells (Figure 2). This progression marks viral infection at the lysosomal compartment showing low pH enzymes activity. There is disruption of the viral molecule, hence releasing the infected nucleic acid and the enzymes essential for viral replication. CQ blocks viral infections necessitating a pH-dependent phase for entry like in the case of Borna virus[22], the minute virus of mice MVMp[23], and the avian

leucosis infection[24]. Interestingly, a pathologist report confirmed that CQ limits the uncoating of the hepatitis A virus, thereby disrupting the whole replication cycle[25].

Replication of Enveloped Viruses Interaction

The enveloped glycoprotein post-translational modification occurs inside the TGN and endoplasmic vesicles and there is a cycling of low pH in glycosyl-transferases and protease. All these processes largely depend on pH, the Mayaro virus progression was evidently inhibited by CQ[26] thereby causing the accumulation of herpes simplex virus 1 particle in the TGN[27]. It was also noticed that in Flaviridae family of protein (flavivirus pr M to M), replication and proteolytic action were inhibited by CQ activity[28]. Therefore, viral pathogenicity is destabilized. Furthermore, chloroquine prompts the manufacture of non-infectious retrovirus particles, observed in HIV-1 and avian reticuloendotheliosis virus REV-An[29]. Inhibition appears to be the mode of action used in the glycosylation of enveloped glycoprotein.

Inhibition of Viral Attachment and Entry in the Host Cell

Firstly, coronavirus establishes a viral particle connection with host cells membrane, through specific cell membrane receptors and the structural protein of the virus. CQ or HCQ could repress the entry of the virus by inhibiting the sialic acid biosynthesis, basic players in the recognition of ligand in the virus-cell. CQ i*n* vitro activity against coronaviruses is due to the restriction of cellular N-glycosylation receptor on the virus r, SARS-CoV-2 and SARS-CoV angiotensin-converting enzyme 2 (ACE2), and perhaps spike in viral protein (S). This leads to a decreased binding activity between cell viral S protein and ACE2

which causes the glycosylation of SARS-CoV S protein in CQ therapeutic doses[30]. In SARS-CoV-2 S protein also undergo glycosylation just like SARS-CoV, however this is seen in novel prospective[31]. During the *in* silico examination[32], it was pointed out that the S protein of SARS-CoV-2 utilizes ACE2 receptor and the sialic acids linked to gangliosides on the cellular surface, potentially enhancing the cellular attachment to the virus. The *in* silico modelling proposes that CQ or HCQ could have high affinity to attach to host sialic acids and gangliosides, potentially inhibiting the interaction between S protein and the plasma membrane in the host. Given these perceptions, CQ or HCQ action could be in two different ways: decreasing the nascent virion infectivity and/or weakening viral passage. CQ was shown to reduce the phosphatidylinositol expression restricting clathrin assembly protein (PICALM)[33], which is a part of the many proteins

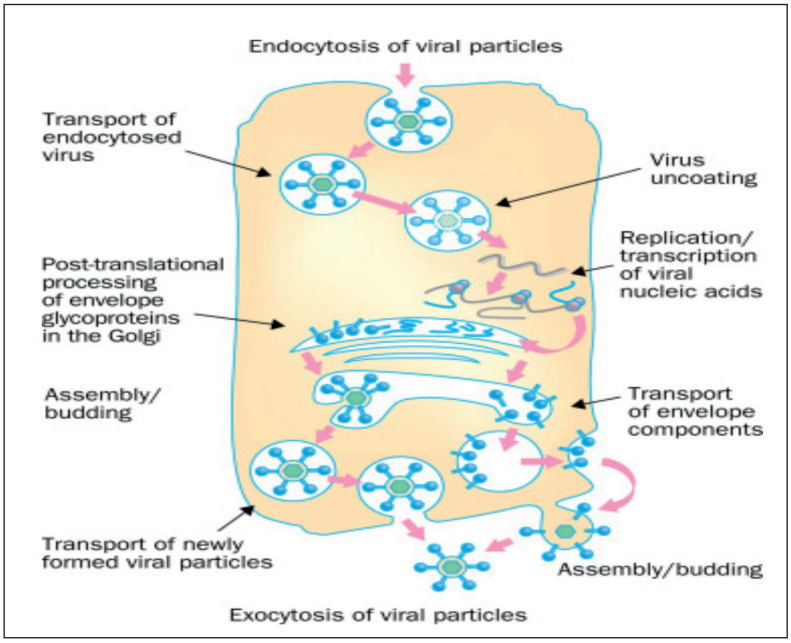

Figure 2. Steps of the replication of various infectious particles that can be targeted by chloroquine (marked by pink rectangles). Chloroquine delays the replication of various viruses either at the early or late phases of viral replication[11].

in clathrin-coated pits that manages clathrin-mediated endocytosis (CME) cell, i.e., an adaptor for cargo selecting, involved in entry of SARS-CoV into the mammalian cells[34]. After the binding of the receptor, coronaviruses S-protein cleaves the endosomal proteases e.g., transmembrane serine protease 2 (TMPRSS2) and cathepsin at the acid dependent site. This allows the endosomal membrane and the viral cell to combine leading to elevated pH thereby causing inhibition. TMPRSS2 and cathepsin move by empowering S protein cleavage of SARS-CoV-2 enzymatic activities[35]. At that point, CQ or HCQ inhibits the entry of virus and attachment in the cell of the host, perhaps causing obstruction of endocytic vesicles viral infection.

Inhibition of Development and Proliferation of Nascent New Viral Particle

CQ or HCQ have antiviral action, after a viral infection. The effect was found in SARS-CoV-2 and SARS-CoV infections *in vitro*[13,14]. Another mechanism could therefore be associated with the activity of the antiviral agent. CQ or HCQ may repress or prevent the fusion of endosome-lysosome membrane prompting the recycling viral receptor membrane, the viral genome and viral uncoating is released into the cytosol after endosomal alkalination, as seen in SARS-CoV[36]. The viral protein developmental processes may be disrupted by CQ/HCQ. This occurs inside the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) and vesicles of trans-Golgi network (TGN) of the cell when there is low pH. Increase in pH could upset proteolytic handling and glycosylation of viral proteins. Similar to S protein, modifications and glycosylation occur in the coronaviruses envelop membrane protein likewise in ERGIC and TGN vesicles because the structural protein in the viruses is numerous[12]. Other cleavage sites apart from TMPRSS2 and cathepsin were recognized in the SARS-CoV-2 S protein that could undergo cleavage by proteases similar to furin. In the TGN there is inhibition of Furin but profound expression is noticed in the lung, its S protein cleavage could be associated with viral progression and proliferation[37]. This could be delayed by CQ, as seen in Chikungunya viral infection[38]. Essentially, their basic properties interrupt compartment of the cell vesicles, CQ or HCQ may equally restrain viral growth. This takes place once the encapsulated virion genomes grow into the ERGIC layers because the virus envelope proteins are embedded, establishing the adult virus[39]. In respiratory disorders, the virus binds with Toll-like receptors (TLRs), causing the deactivation of mitogen-activated protein kinase (MAPK) pathways, especially p38 MAPK. The respiratory system viruses inhibit the MAPK activation, causing the activation of their downline targets to transmit their proteins for virus progression and spread. The above mentioned effects are very significant in individuals having underlying airways diseases, similar to constant obstructive pulmonary disease (COPD) or asthma. These individuals have viral respiratory disease and CQ was found to interrupt the p38 MAPK activation pathway usually turned on and activated[40] subsequently inhibiting viral infection[41,42]. COVID-19 individuals with COPD could benefit from the inhibitory activity because their SARS-CoV-2 infection could be life-threatening. In this way, other than viral connection, passage and uncoating, genome discharge, protein developmental cycle and assembly of nascent viruses development and progression might be restrained by the simple drug, resulting in a diminished infectivity.

Recent Findings on the Activating Mechanisms of CQ or HCQ in SARS-CoV-2 Infection

There is insufficient information about the mechanism of CQ or HCQ activation in the management of SARS-CoV-2 disease. Past publication on the ability of CQ OR HCQ in this infection was proved by *in vitro* studies. Wang et al[13] revealed the ability of CQ to inhibit SARS-CoV-2 disease (myriad of infection, MOI = 0.05) in Vero E6 cells at decreased micromolar concentration (EC50 = 1.13 μM and EC90 = 6.90 μM, 2 days), medically accessible using 500 mg daily administration. Its effectiveness was assessed through the evaluation of virus copies in the cellular surface using Western blot examination, immunofluorescence microscopy, RT-PCR, accessing the nucleoprotein (NP). The scientists noticed that CQ disease inhibited the entry and post-entry points (drug administered 120 mins before the management of the virus, Western blot was done 14 h later). The investigators also analyzed CQ and HCQ efficacies inside a similar cell prototype and noticed that cell toxicity between the two prescriptions showed non significant differences. Estimating antagonistic effect to viral viability at various MOIs (0.80 0.2, 0.02, 0.01) using RT-PCR lower E50 values for CQ at all MOIs (CQ has 7.36, 7.14, 3.81, 2.71 for and HCQ 12.96, 17.31, 4.06, 4.51)

was noticed, affirmed via immunofluorescence examination for NP.[14] These outcomes proposed that CQ is possibly more suitable than HCQ. The researchers also affirmed that HCQ and CQ at the entry and post-entry point brought about double activity in HCQ. another study projected that the cellular barrier of discovered virions and viral particles mostly in early and late endosome-lysosomes (EE, LELs) were under regulated conditions. At the point of cell treatment with CQ and HCQ, the EEs showed more particles, pointing the action of the drugs to block endocytotic vesicle intermediate stage development and possibly slowing down the EEs to LELs viral transportation, noticed a significant advancement in the viral genome movement. The researchers observed that the CQ and HCQ drug causes abnormal development of EEs; however, HCQ extended the number and size of LEL and the administration of CQ did not activate any modifications in LELs number and size. By the way, the structure of the vesicle was disturbed, proposing partially opposite modes of activity in both drugs. The action of CQ and HCQ against SARS-CoV-2 activity *in vitro* was affirmed looking at similar cell model[43]. These writers discovered EC50 estimates of about 5.47 and 23.90 for CQ and 0.72 and 6.14 mM on days 2 and 1 CQ and HCQ independently. This is however different from the past report. HCQ had a better action. Both drugs have increased activity against viral infection during administration prior to virus infection, EC50 estimates of >100, 6.25 and 5.85 mM for HCQ and 18.01 for CQ individually on days 1 and 2.

The Antagonistic Action of CQ on SARS-CoV-2

Due to the said wide range of its anti-viral activity on most viruses, particularly in SARS-CoV- upon the entry of COVID-19 into the cell occurs via endolysosomal pathway[44], it seemed appropriate to examine the potential anti-SARS-CoV-2 activity of CQ. Both CQ and remdesivir (antiviral drug) have recently been reported to repress SARS-CoV-2 *in vitro* and in humans experiencing COVID-19[13]. The CQ and HCQ activity was assessed on some enveloped viruses and immune activation. Suggestions were made that the drug could be useful for SARS management in the treatment center. As of now, common antiviral drugs particularly oseltamivir and ribavirin have not been shown to be effective in SARS treatment. Corticosteroids could be advantageous in controlling the lung inflammatory reactions[45] but unrestrained immunodepression could cause pulmonary distress. Lately, SARS causative agent has been described as novel corona virus[46]. Recent examinations support the possibility that corona viridae attacks target cells using endocytic pathway and the replication can be restrained by chloroquine[47]. Human Covid HCoV-229E infected cells given nocodazole (a microtubule depolymerising specialist which limits transport from early to late endosomes) secreted HCoV-229E antigens in small quantity[48]. The outcome demonstrates that in HCoV-229E disease, there is a need for transportation in the endosomes. HCoV-229E antigens reduced in chloroquine treated cells[48]. Recently, the China National Center for Biotechnology Development pointed out that CQ among three drugs has a favorable profile against the novel SARS-CoV-2 which causes COVID-19. In provinces around China especially in Beijing Clinic, Guangdong province CQ was employed. According to the initial report[49,50], CQ was found to cause reduction in fever including improved lung tomography in about 100 infected patients and required more chances to recover when compared with the control group. Moreover, no antagonistic effects were noticed and therefore the Chinese Medical Advisory Board has recommended that in the treatment of SARS-CoV-2, CQ could be employed. Thus, CQ is probably the primary drug utilized abroad and China is in the front line fight against SARS-CoV-2 disease. Despite the wide use of chloroquine in the treatment of malaria, it is important to take note of the safety dose, the side effect is macular retinopathy[51], another side effect of CQ and HCQ could be cardiomyopathy[2,53] A SARS-CoV-2 infected patient profile must take note of the adverse effects of chloroquine treatment . Notwithstanding, chloroquine right now is one of the best drugs used in tackling the severity of SARS-CoV-2 diseases in people. HCQ, an analogue of chloroquine has little observable drug-drug interactions. In the past SARS outbreak, HCQ was thought to have adverse effect on SARS-CoV *in vitro*[54]. It therefore means that HCQ might bring a ray of hope in COVID-19 disease treatment. Nonetheless, until this point, there is no hypothetical proof pointing to HCQ utilization as SARS-CoV-2 disease treatment. Other mechanism of action of HCQ and/or CQ is yet to be completely explained. Past researches discoveries have recommended that HCQ and CQ repress the COVID-19 progression. Medications can alter the pH of the cell layer surface subsequently hindering the viral infection

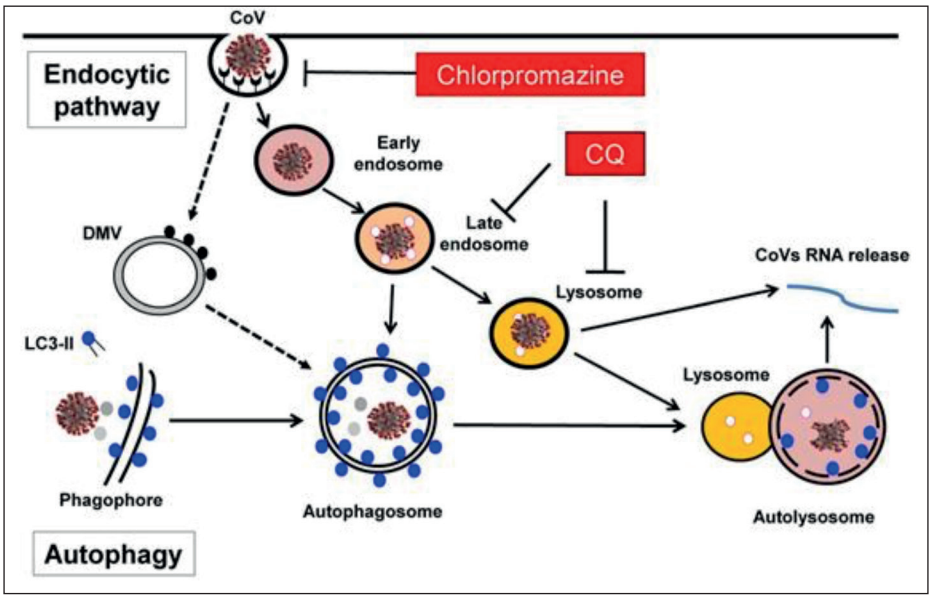

Figure 3. Diagrammatic representation of chloroquine effect on the replication cycle of severe acute respiratory coronavirus 2 (SARS-CoV-2).

from affecting the cell membrane. It also hinders nucleic acid duplication, viral proteins glycosylation, assembling of virus, nascent virus molecule transport, viral discharge, and different cycles to accomplish its antiviral effects[55]. In certain patients reports, the immune response to the SARS-CoV-2 infection results in elevated cytokines interleukin (IL)- 6 and IL-10[56,57]. This may increase cytokine storm, followed by multiorgan disruption and possibly death. Both CQ and HCQ have immunosuppressive effects and can reduce increased immune response[11,58]. With this in mind, it is possible that early treatment with both medications may help inhibit the deadly progression of the disease progression. In chronic SARS-CoV-2 patients, corticosteroids administration might be injurious, if used they suppress the immune system and increase infection[59] (e.g., tocilizumab) [60].

Functional Principle of Chloroquine

CQ has various operating principles that may vary as shown by the researches conducted on microorganisms. CQ could hinder an initial viral cycle by meddling with viral particles binding to their cell surface receptor (Figure 3). CQ appeared to repress quinone reductase 2[61], an underlying neighbor of UDP-N-acetylglucosamine 2-epimerases[62] associated with the biosynthesis of sialic acids. The sialic acids are acidic monosaccharides found at the end of sugar chains present on cell transmembrane proteins and are the basic components of ligand recognition. The possible inference of CQ with sialic acid biosynthesis could be a pointer to the wide antiviral range of that drug since infections, for example, the human HCoV-O43 and the orthomyxoviruses use sialic acid moieties as receptors[63]. The strength of anti-SARS-CoV-1 effects of CQ *in vitro* was ascribed to a deficiency in the glycosylation of a virus cell surface receptor, the angiotensin-converting enzyme 2 (ACE2) on Vero cells[64].

CQ could also impede the initial phase of virus replication by meddling with the pH-dependent endosome-mediated viral entry of enveloped viruses, e.g., Dengue or Chikungunya virus[65,66]. Due to the alkalization of endosomes, CQ was a compelling *in vitro* treatment against Chikungunya infection after additional exposure to Vero cells prior to virus exposure[67]. The mode of inhibition is probably the inhibition of endocytosis as well as fast rise of the endosomal pH and repeal of infection endosome fusion. A pH-dependent mode of entry of COVID-19 into target cells was also revealed for SARS-CoV-1 in the binding of the DC-SIGN receptor[68]. The stimulatory step happens in endosomes at acidic pH resulting in fusion of the viral and endosomal layers prompting the delivery of the viral SARS-CoV-1 genome into the cytosol[36]. During the lack of antiviral medication, the virus targets the lysosomal compartment with low pH, the enzyme's activity disturbs the viral molecule, subsequently releas-

ing the infectious nucleic acid, vital for its replication[69]. CQ-mediated the inhibition of hepatitis A viral infection was discovered to be related to uncoating, impeding its whole cycle of replication[23]. Post-translational modification of viral proteins is also disrupted by chloroquine. These post-translational modifications, which include proteases and glycosyltransferases, occur inside the endoplasmic reticulum or the trans-Golgi network vesicles and may require a low pH. For HIV, the antiretroviral impact of chloroquine is inferably from a post-transcriptional inhibition of glycosylation of the gp 120 envelope glycoprotein and the neosynthesised viral particles are not infectious[70,71].

CQ also hinders the replication Dengue-2 infection by influencing the normal proteolytic processing of the flavivirus prM protein to M protein[28]. Therefore, viral infectivity is weakened. Examining the herpes simplex virus (HSV) model, CQ repressed growth of group of non-infectious HSV-1 particles in the trans-Golgi network[27]. With non-human coronavirus, it was demonstrated that the intracellular site of COVID-19 spread is dictated by the limitation of its membrane M proteins that gather in the Golgi complex beyond the virion budding site[72], proposing that the potential activity of CQ was observed at the cycle of replication in SARS-CoV-2. Recently, it has been announced that the MERS-CoV M Protein C-terminal space contains a trans-Golgi network limitation signal[73]. The action of CQ was also noticed in the disruption of the viral developmental process, viral protein by pH regulation[28] and viral antigen recognition by dendritic cells and the necessary endosomal acidification through a Toll-like receptor-dependent pathway[74]. Unexpectedly, other proposed CQ effects on the immune system comprise elevated soluble antigen export into dendritic cell cytosol and the improvement of cytotoxic $CD8^+$ T-cell reactions against human antigens in virus[75]. The influenza virus model showed that chloroquine improved non-replicating viral antigen in the cross-presentation of dendritic cells to $CD8^+$ T-cells employed to lymph nodes depleting the site of infection, evoking a comprehensively defensive resistant reaction[76]. The action of CQ on the immune system could also be via cell signaling and regulation of pro-inflammatory cytokines.

CQ represses phosphorylation of the p38 mitogen-initiated protein kinase (MAPK) in THP-1 cells just as caspase-1[77]. Activation of cells by means of MAPK signaling is always necessary for infections to accomplish their replication cycle[78]. In the model of HCoV-229 COVID-19, CQ-induced infection inhibition happens through inhibition of p38 MAPK[41]. CQ is a notable immunomodulatory agent fortified as an intermediate for anti-inflammatory reaction[11]. The use of this drug has also been observed in the treatment of rheumatoid arthritis, a well-known inflammatory disease, including others like[79-81] lupus erythematosus[82,83] and sarcoidosis[84]. CQ represses the mRNA expression of interleukin-1 beta (IL-1β) in THP-1 cells and lessens IL-1β delivery, macrophage/monocytes IL-1 and IL-6 cytokines tumor necrosis factor-alpha (TNFα) production. Another report[90] showed the inhibition of the TNFα receptor in U937 monocytic cells during CQ treatment. In the Dengue virus model using U937 cells infected, CQ inhibited TNFα, interferon-alpha (IFNα), IFNβ, IFNγ, IL-6 and IL-12 gene expression[91] .

SARS-CoV-2, similar to some coronaviruses in humans, contains three envelope proteins, the spike (S) protein (180-220 kDa), the layer (M) protein (25-35 kDa) and the envelope (E) protein (10-12 kDa), necessary for infectious virions entry into target cells. The virus contains the nucleocapsid (N), an important part of replicase complex needed for binding to viral genomic RNA, and nsp3. Hemagglutinin-esterase (65 kDa) is a subset of β-corona viruses used in sialic acids binding at the glycoprotein surface. The host tropism determines S glycoprotein. SARS-CoV-2 also binds to angiotensin-converting enzymes protein 2 (ACE2) expressed on pneumocytes[92]. When ACE2 is bound, conformational changes are triggered on S glycoprotein orchestrating cleavage of the S protein by transmembrane protease TMPRSS2 and S protein release into the cell supernatant leading to antibodies neutralization[93]. There is movement of cellular virus through the early and late endosomes, cleavage of protease cathepsin L by S protein at low pH, subsequently leading to viral envelope fusion with phospholipidic endosomal membrane bringing about viral genome fusion into the cell cytoplasm. Replication begins, followed by the translation of positive-strand viral genomic RNA to a negative RNA strand needed for virus mRNA synthesis template. There is a rise and then fall in the synthesis of negative MRNA strand relative to that of the positive strand. In infected cells, more positive than negative strands are observed (around 10 and 100 times more). The infected cell ribosome machinery is diverted particularly for the virus, which synthesizes its non-structural proteins (NSPs) which are

collected in the replicase-transcriptase complex to support viral subgenomic mRNA synthesis[12]. After replication, there is translation of the envelope proteins, insertion into endoplasmic reticulum followed by transportation into the Golgi compartment. Before the formation of matured virions, there is viral genomic RNA arrangement into the nucleocapsid and fusion of envelope proteins at the budding stage. The M protein interacts with other viral protein causing the localization of trans-golgi network, before viral assembly. After assembly, the nascent viral particles are moved to vesicles cell surface and are delivered by exocytosis. Possibly, chloroquine meddles with ACE2 receptor glycosylation, subsequently stopping the binding between the target cell and SAR-CoV-2. Sialic acids biosynthesis is inhibited by chloroquine. This may be necessary for SARS-CoV-2 to bind to the surface of the cell. The successful binding of viral particles leads to regulation of endosome acidification by chloroquine causing restriction of both autophagosome development and viral replication caused by MAPK cell initiation reduction; chloroquine may likewise restrain viral replication. M protein development, virion assembly and budding can be disrupted by chloroquine. This was also part of the effects of chloroquine seen on the immune system[11].

ER-Golgi moderate compartment (ERGIC), the report exhibiting chloroquine exceptional capacity in lessening viral replication, with an Effective Concentration (EC) 90 of 6.90 μM was attainable with standard dosing, because of its infiltration in tissues, as well as in the lung[13]. The researchers pointed out that chloroquine disrupts viral diseases by elevating endosomal pH and reducing glycosylation of cell receptor in SARS-CoV. The authors inferred that the antiviral effect *in vivo* could be a result of its established immunomodulatory effect[13].

Studies

The Dutch Center of Disease control (CDC), in a public record on the internet, proposed that the treatment of extreme infections requires admission to the clinic with oxygen treatment or admittance to the ICU with CQ[94]. Notwithstanding, the record also expressed that treating patients just with ideal steady consideration is still a sensible choice, due to the absence of strong evidence. The recommended treatment in adults includes CQ base (6 tablets A-CQ 100 mg; 600 mg) monitored by 300 mg following 12 h on day 1, at that point 300 mg × 2/ on per of on 2-5 days. This record also underlined the requirements for halting the treatment at day 5 to diminish the risk of side effects, bearing in mind the drug long half-life (30 h) and the need to separate regimens dependent on CQ phosphate and CQ base since 500 mg of the first set to 300 mg of the second[94]. Another rule recorded by the Italian Society of Infectious and Tropical illness (Lombardy area) suggests the utilization of CQ 500 mg × 2/ or HCQ 200 mg die for 10 days, despite the fact that the treatment may differ from 5 to 20 days as per clinical gravity. The recommended target populace went from patients with mild respiratory indications and comorbidities to patients with serious respiratory distress[95].

Chloroquine and Hydroxychloroquine as Antivirals in COVID-19: A Role for Hemin/Hemoglobin

The CQ and HCQ work differently against virus and Plasmodium. The main differential factor is the hemin/hemoglobin. Against virus like SARS-CoV-2, they work by diminishing the efficiency of the virus at entering host cells and decreasing its replication rate. In malaria, they work by poisoning the parasite digestive system (vacuole) via increasing free hemin that is toxic through oxidative stress and enzyme inhibition (cysteine proteases and others). The targeted cells are also different: ACE2-expressing cells such as epithelial ATII cells and other extra pulmonary cells in SARS-CoV-2 infection and red blood cells in malaria. In both circumstances, cell systems where CQ and HCQ accumulate are involved: lysosomes and related acidic compartments of host cells in SARS -CoV-2, and digestive vacuole in malaria parasite. It is thought that CQ and HCQ exert their anti-viral actions mainly via endosomes. However, the main entry point of SARS-CoV-2 is not by the endosome but instead by spike S protein. This may suggest that additional mechanisms could be involved in their antiviral action. Most drug candidates investigated in COVID-19 target key proteins of the host cell or the virus. The host's cell proteins are TMPRSS2 protease or cathepsins (used for virus entrance) and enzymes ACE2 (virus receptor). The targeted viral proteins are the S protein (a glycoprotein), the RNA-dependent RNA polymerase (RdRp), and the two proteases that are essential for viral replication: main protease (3Clpro), a chymotrypsin -like cysteine protease, and papain-like cysteine protein (PLpro). The 3CLpro utilizes a Cys/His catalytic dyad whereas PLpro has a classical Asp/His/Cys catalytic triad[96]. PLpro possesses a C-terminal

zinc-finger like domain bearing four cysteine residues that are critical for catalytic function[96]. Drugs are being tested as inhibitors of these cysteine proteases in order to block viral replication. CQ is not a good inhibitor of cysteine proteases. However, it interacts with hemin and increases oxidative reactions (oxidative stress), as well as inhibition of cysteine proteases[97]. These events might hypothetically occur in the host and virus during infection. Cell cultures might contain (methemoglobin) and hemin eventually influencing the antiviral action of CQ and HCQ residual hemoglobin. *In vivo*, hemoglobin is confined into red blood cells, which in contrast to Plasmodium are not presumably targets of SARS-CoV- 2. Recent results suggest that the virus might use CD147 receptor as an additional gate into host cells and the same receptor is used by Plasmodium to enter into red blood cells[98,99]. However, noticeable hemolysis is not stated as a common feature of patients with COVID-19 although ferritin appears to increase. Nevertheless, there are possible sources of extravascular local cell- free hemoglobin and hemin that should be considered. Hemoglobin could be locally released from red blood cells breakdown in capillaries close to alveolar cells damaged during infection. Thus, SARS-CoV infections caused hemorrhages in the alveolar space of the lungs[100], and lung hemorrhages in animal models infected with coronaviruses are significantly reduced with antivirals[101]. SARS-CoV-2 attacks blood vessels and some patients show coagulation dysfunction and develop blood clots while others develop pulmonary embolism. The pathological analysis of the lungs of COVID-19 patients shows noticeable microvascular thrombosis and hemorrhage linked to interstitial inflammation and infected alveolar[102].

Clinical pathological investigations of lung biopsies and dissections of COVID-19 patients demonstrate alveolar discharge along small vessel clots arrangement around the lung periphery[103,104]. Alveolar Hemorrhage is a typical component of the acute respiratory distress syndrome (ARDS), a condition related with COVID-19. ARDS patients have raised degrees of cell-free hemoglobin inside the alveolar space from red platelet breakdown[105], and it contributes to the intensity of lung pathogenesis. Cell-free hemoglobin caused elevated inflammation, injury to the cell epithelial and loss of integrity of alveolar capillary barrier[105]. It converts methemoglobin and delivers free Hemin[106] cellularly.

Hemin increased alveolar-capillary permeability (barrier dysfunction) but did not cause alveolar inflammation or epithelial cell injury in the lungs[105]. Finally, the drugs CQ and HCQ might also induce erythrocyte break down in some patients[107]. Extracellular hemin/hemoglobin released from red blood cell breakdown might be a target for the action of CQ and HCQ. On the other hand, hemoglobin is expressed in blood cells other than erythrocyte such as epithelial cells and activated macrophages. Expression of this protein has been observed in several human tissues including the lungs, particularly in alveolar epithelial cells type II (ATII) that produce the pulmonary surfactant essential for lung function[108-110]. Hypoxia greatly increased hemoglobin expression in ATII cells, and decreased surfactant proteins[110]. Hemoglobin in these cells could increase the concentrations of oxygen, and oxygen transport facilitation across air-blood barrier[110], and it might have a role in ARDS. Remarkably, epithelial ATII cells that are main targets of SARS-CoV-2 infection also express hemoglobin, and this expression highly increases during hypoxia, a phenomenon occurring during viral infection. Hemoglobin/hemin expressed in ATII cells might be a target for the action of CQ and HCQ. Local extracellular hemoglobin or hemoglobin expressed in ATII cells could be oxidized to methemoglobin (Fe^{3+}). This oxidation produces reactive oxygen species (ROS) (H_2O_2), in the presence of inflammatory-cell-derived oxidants[111]. Methemoglobin is toxic, generates ROS, and releases free hemin[112], which can be incorporated into endothelial cells[111,113] and perhaps other cells as well[114]. Endothelium protects itself from heme/hemin by heme induction, heme oxygenase degradation and production of large amounts of ferritin, iron binding protein[113]. CQ and HCQ could modulate those actions. For example, their interaction with free hemin could trigger cysteine proteases inhibition and oxidative reactions of the virus and the host, which could result in antiviral actions.

CQ could also limit degradation of extracellular hemoglobin by alveolar macrophages. During disposition of extracellular hemoglobin in hemoglobin excavenger receptor CD163-positive HEK cells, CQ action resulted in intracellular hemoglobin trapping compromising clearance and abolished expression of heme oxygenase-1 (HO-1). CQ blocks the elimination of hemoglobin (or methemoglobin) by interfering with lysosomal hemoglobin degradation, and caused intracellular accumulation of heme and globin[114]. The results support previous studies stating that chloroquine acts by increasing free hemin (and hemoglobin) to potentiate oxidative reactions (oxidative stress)

and inhibition of cysteine proteases[97] that might affect the host body and virus. However, for effectiveness, this mechanism would need the presence of sufficient local free hemin/hemoglobin to interact with chloroquine. It is currently unknown whether this really occurs during infection in COVID-19. In a recent preprint[115], it is reported that non-structural proteins of SARS-CoV-2 could attack hemoglobin and heme to release porphyrins used for the virus. This could be interpreted as the virus degraded hemoglobin. However, this is a bioinformatics study and not an experimental work, and does not prove the breakdown of hemoglobin or heme to porphyrins, that would need a kind of heme oxygenase. As described here, CQ and HCQ exert diverse biological effects as shown also by their anti-inflammatory actions in autoimmune (lupus), rheumatoid arthritis diseases and in porphyria[116117]. As discussed here, an interaction of these drugs with heme is expected. In this regard, intravenous hemin (hydroxyhemin or hemin arginate) is used for the treatment of acute porphyria attacks. It helps to overcome the relative deficiency of heme in the liver and reduces the supply of porphyrins and precursors needed for heme production. During the current COVID-19 pandemic, porphyria patients could be infected with SARS-CoV-2 and still be treated with hemin for-patients/COVID-19-and-porphyria). Harmful drugs are avoided during treatment with hemin although CQ and HCQ might probably be safe based on this evidence though they are still insufficient. So far, it is unknown whether possible simultaneous cases of acute porphyria attack and COVID-19 have been treated with hemin, CQ and HCQ, but it would be of interest to know the outcome because an interaction of hemin with these drugs is expected. Remarkably, hemin exhibits antiviral actions against several viruses such as HIV, Zika, influenza, or Ebola. This action could be related with the induction of heme oxygenase enzyme[118]. Therefore, the antiviral action of hemin supports a possible mechanism for antiviral action of CQ and HCQ with involvement of hemin, as discussed above. Hemin inhibited HIV replication *in vitro* and reduced viral load in mice[119]. It significantly inhibited replication of Zika virus *in vitro*[120]. Hemin showed anti-influenza virus activity *in vitro* and *in vivo*[121]. It ameliorated influenza pneumonia and attenuated virus-induced lung injury, lymphocytopenia and local inflammation in a mouse model. Hemin protected mice from death and body weight loss caused by influenza A virus infection[121]. Hemin had an important effect in the reduction of Ebola virus replication. Treatment of different human cells with hemin reduced infection by >90% and showed minimal toxicity to infected cells[120]. This inhibition could be partly due to heme oxygenase (OH-1) activity and expression. The activity of reverse transcriptase in murine leukemia virus was repressed by hemin and inhibition increased 1000-times when hemin solution was aged[122]. Hemin also potentiated the activity of artemisin in against Hepatitis C virus showing synergistic effects[123]. Taken together, the antiviral effects of hemin are noticeable and could be beneficial in the present situation of absence of effective treatments in COVID-19. In this regard, the action of hemin, and the combined action of hemin and CQ and HCQ may be worth testing in SARS-CoV-2 (COVID-19) studies evaluating antiviral action and effects on the disease. Nevertheless, possible safety and toxic effects should also be considered as hemin may induce toxic actions[124] and CQ and HCQ are not exempted from the undesirable effects such as heart problems and retinopathy[107,125].

CQ/HCQ and Iron Metabolism: SARS-CoV-2 Infection and the Role of Iron

The fundamental component for every living being is iron which is also due to its activity in redox reaction l as a basic cofactor in many enzymatic and proteins associated with essential cell function like ATP production, replication and transcription of DNA. Indeed, most viral infections also need iron, as they depend on metabolic mechanism of the host for genome replication and production of mRNAs for translation into useful viral proteins[126]. Subsequently, the cellular repletion of iron increased viral replication and decreased (this term has been moved, please check if it is ok) multiplication or progression can disrupt the life cycle of the virus.

In the course of infections and inflammation, pro-inflammatory cytokines cause iron deficiency. A good number of these directly influence the stability of iron similar to Interleukin-1β, Interleukin-6 and Tumor Necrotic Factor-α. These cytokines, especially interleukin-6, bring about iron-regulatory hormone hepcidin (HAMP) upregulation, basically delivered by hepatocytes into the circulatory system direct the activities of iron homeostasis systematically. Iron transportation is blocked in the cell by systemic HAMP blocks through ferroportin 1 (FPN1), bringing about decreased iron absorption in the intestine, elevated macrophages and hepatocytes iron retention and

at last inflammatory/infection anemia[127]. Some cells apart from the hepatic cells have yielded and delivered HAMP that circulate as autocrine and paracrine particles, maintaining a constant internal environment for iron[127]. Not just cells of the immune system like monocytes, lymphocytes, and macrophages (together with alveolar macrophages) but also the airways epithelial cells have been shown to yield HAMP in the course of inflammation, infections and possibly aggravated lung injury[128]. The peptide HAMP is associated with the critical phase protein and natural antimicrobial resistance[129], lactoferrin (LF), transferrin (Tf), ferritin (FT), hemopexin (HPX) and haptoglobin (HP) are proteins strongly associated with iron, viral diseases Investigations have been made on viral diseases and iron breakdown in humans[130].

Incitation of Iron Starvation by CQ OR HCQ in the Cell: A SARS-CoV-2 Duplication Mechanism of Action

Several models of investigation showed that iron entry was restricted by CQ like in eukaryotic model Saccharomyces cerevisiae, CQ distorted iron uptake causing competitive inhibition of iron entry and starvation. Yeast that lacked iron showed elevated sensitivity to CQ by the methods of knocking out genes associated with uptake of iron or by utilizing iron chelators[131]. In the cells of mammals, CQ acts similarly by inhibiting endocytotic complex of Tf/transferrin receptor 1 (TFR1). The fibroblast obtained from cultured rats gave key evidence that Tf uptake was inhibited by CQ[132]. The major plasma iron transporter is Tf that maintains the cofactor in a passive redox environment by circulating it across human body cells. Two Fe^{3+} firmly binds Tf. TFR1, found on most cell types in the plasma membrane, binds and internalizes Tf into endocytic vesicles through CME[133]. Similar to the previous ones, CQ has been shown in treated mice macrophages to lessen the expression of PICALM[134]. This protein is expressed ubiquitously with CME and its inadequacy was shown to bring about abnormal metabolism of iron and anaemia in mice[135] for embryonic fibroblast in murine iron starvation, elevated expression of surface TFR1 with low iron level intracellularly[136]. At that point, CQ OR HCQ administration may bring about cellular iron starvation and inhibition of the uptake of Tf/TFR1 complex. Iron release from Tf after iron-loaded Tf/TFR1 complex CME and its cytosolic movement is a basic stage for cell procurement and further utilization of iron. The endosomal acidic millaeu delays Tf binding to Fe^{3+}, leading to the vesicular delivery[133] of CQ or HCQ, elevating endocytic vesicles pH, potentially repressing endocyric vesicle Tf iron expulsion. Inside the vesicle of the endocytes, metalloreductase reduces transported Fe^{3+} to Fe^{2+} a six-transmembrane epithelial antigen of the prostate 3 (STEAP3) moved into the cytosol from the lysosomes by mucolipin 1 (TRPML1/MCOLN1), different carriers and divalent metal-ion transporter 1 (DMT1)[133]. The transportation of iron from DMT1 is pH dependent, particularly active when the pH is low. Before iron can be moved into the cytoplasm by DMT1 endosomes, an H^+/Fe^{2+} symporter needs a positively charged electrochemical potential gradient[133].

Na^+, K^+ and Ca^{2+} are ions that can penetrate TRPML1/MCOLN1 channel also transporting Fe^{2+}. Before the delivery of cation to the cytosol from the lumen, there must be late endosome, acidic vesicles, lysosome and cation delivery[130]. At that point iron delivery from endosomes into the cytosol is inhibited by CQ or HCQ.

CQ or HCQ is used to weaken autophagic movement and the fusion of endosome or lysosome[137]. The basic cellular iron storage is FT which compartmentalizes the non-reactive form of iron until it is used. The delivery of iron from FT mostly happens by protein break-down through a specific lysosome-autophagy pathway called ferritinophagy[130] in which CQ is inhibited[138].

Additionally, both TRPML1 and DMT1 have been involved in the iron arrival from ferritinophagy, this occurs in acidic environment because these channels open to allow trapping of autophagic vesicles. The above-mentioned steps cause cellular iron deprivation. The aforementioned condition may perhaps affect the life cycle of SARS-CoV-2, though presently the experiments have not been proven because this pandemic is a strange disease, hence the need for more study. ACE2 receptor is the by which SARS-CoV-2 enters the cell in humans, especially in the lung alveolar epithelial cells, vascular endothelia, skin, kidney, heart, muscle and bronchial and gastrointestinal system[139]. Generally, in human cells iron is an important cofactor for SARS-CoV-2 target cells and many other important life processes involving proteins such as cell development, bioenergetics, nucleic acid synthesis including the virus life cycle. Some viral diseases are found to elevate the uptake of iron in cells, the inhibition of iron starvation during cell cycle of viruses has been proven in various human infections like Hu-

man Deficiency Virus 1 (HIV-1), Human Cytomegalovirus (HCMV) and hepatitis C infection (HCV)[126]. Elevated uptake of iron in cell including FT synthesis has been exhibited in infected mice liver with mouse hepatitis virus type 3 (MHV-3), from the family called Coronaviridae[140].

CQ or HCQ Could Prompt Iron Starvation in the Cell: A Possible Valuable Equilibrium Between Innate And Adaptive Immune Response

Another significant point noticed about CQ or HCQ is their action on cellular immunity through iron starvation, considering the natural and adaptive immune responses against viral infections. For activation, proliferation and proper immune functions, iron is an essential requirement. Excess iron frequently brings about delayed immune response to infections, as seen in hemochromatosis (HH) or thalassemia patients[141]. Therefore, immune responses that are extreme or dysregulated have specific significance in COVID-19 pathogenesis[142] in the same way as other coronaviruses[143,144]. Many coronaviruses have shown direct infection to both their inborn and adaptive cellular immunity[143,149]. At that point iron starvation could equally repress the infections mentioned earlier. Native macrophages can differentiate under the cytokines stimulation in customarily initiated pro-inflammatory macrophages (M1), stimulated by IFN-γ, TNF-α or IL-4 and IL-13, otherwise activated macrophages (M2), associated with microbe clearance, repair of tissue and decreased inflammation. However, M2 macrophages possess low levels of iron; M1 macrophages retain iron, secrete significant levels of pro-inflammatory cytokines, free radicals, HAMP to kill microbes and limit the exit of iron.

Elevated deposition of iron inside the macrophages causes polarization of M1, the pro-inflammatory state elongation as a result early switching to the M2 state[146]. The life cycle of viruses can thrive intracellularly by retaining iron in the macrophages during infection advancing the inflammatory process to that eventually bring about antagonistic effects. Furthermore, secondary diseases with different microbes could be caused by excess iron in macrophages. Prolonged administration of CQ brings about reduced iron content in rat models[9,147] indicating CQ capacity to lessen iron content in liver and spleen and alveolar macrophages of rats groups in the control, iron-loaded and iron-deprived rats. Also, rats group given lipopolysaccharide (LPS) showed decreased oxidative reaction, estimated by discharge of NO_2, after CQ treatment suggesting that CQ may avert infections, especially those related or caused as a result of iron overload, by restricting iron accessibility in the cells affected by infection similarly in macrophages, thus decreasing inflammation. In any case, homozygous and heterozygous mutations in hemochromatosis gene (HFE) decrease the effect of CQ on iron expulsion in porphyria cutanea tarda[148]. Another model is murine, CQ appeared to lessen macrophage activation syndrome especially in hemophagocytic condition by the initiation of pristane, phagocytic functions, by decreasing the penetration of macrophage, production of cytokines and causing a decrease to FT lactate dehydrogenase and triglyceride levels[149].

For activation and proliferation, immune cells need iron but excessive iron is proven to impede the antigen production. The introduction by APCs alters $CD4^+$ cells and modifies $CD4^+/CD8^+$ lymphocyte proportion, increases circulating B cell produced by immunoglobulins, decreases natural killer (NK) cell has the ability to lyse cells and disturbs supplement initiation[150]; however, iron deficiency produces immunosuppressive effects to T cells[151]. At that point, CQ or HCQ administration, due to their activity on iron homeostasis, might have a wide function in SARS CoV-2 adaptive response regulation.

CQ OR HCQ Reduces IL-1β, IL-6 and TNF-α Release: Could Possibly Reduce the Release of Local and Systemic HAMP

CQ and HCQ inhibit cytokine, through their action on iron homeostasis, inflammation reduction and reestablishment of initial iron homeostasis, thus protecting erythropoiesis. Furthermore, IL-1β, IL-6, TNF-α induce HAMP release both locally and systemically. Iron is fundamental to life, however excess iron is harmful, due to the redox potential that hinders oxidative stress that is detrimental to essential cell constituents. Iron accessibility is then firmly controlled equally systemically and cellularly[126,130,153,154]. On the other hand, deficiency of iron hinders its appearance, using the hepatic cells and macrophages in the intestine to cause the absorption, maintenance and discharge of iron. Erythropoietin (EPO) as well as hypoxia downregulate HAMP expression through

erythroferrone (ERFE) that permits the recruitment of iron for the formation of red blood cells, but during inflammation all these are upregulated. IL-6 initiates HAMP during inflammation, via the JAK/STAT3 pathway and BMP/SMAD pathway, yet in addition TNF-α and IL-1β have a direct function on the regulation of HAMP[155-157]. As expressed above, CQ or HCQ does not just interfere with iron initiation in the cell causing its starvation in alveolar macrophages[9,147] bringing about a change to M2 mitigating state, it also restrains, TNF-α, IL-1β and IL-6 release, perhaps decreasing native HAMP liberated through the macrophages. This decrease can bring about more reduced retention of iron in these cells permitting inflammation resolution. Additionally, cytokines reduction might bring about the decrease of HAMP in the system, through elevated iron absorption in the intestine, enhancing anemic infection. Interestedly, COVID-19 signs have been abated by EPO treatment[158]; however, by *in silico* modelling, SARS-CoV-2 now plausibly binds heme causing the dissociation of porphyrin and iron[159].

The Administration of CQ or HCQ for Treatment, Reduces Thrombosis, Anemic Infection by Reducing HAMP and Releasing

Further investigations linked thrombocytic events with thrombocytosis induced by iron deficiency anemia (IDA)[160-162]. A new report proved that IDA patients showing thrombocytosis had twice as much tendency of developing thrombosis than IDA patients with normal platelet count[163]. Remarkably, patients diagnosed with COVID-19 with serious pneumonia seem to have elevated platelet count compared to patient with severe pneumonia without COVID-19[164]. This kind of elevation is predominant among the non-survivor compared with COVID-19 patients that are survivors[165]. Animal models like Sprague-Dawley are used to explain the influence of iron deficiency on thrombotic susceptibility induced by thrombosis. They were fed with an iron-free diet, while the ordinary diet regimen served as control.

Thrombocytosis caused by iron deficiency and platelet count resulted in a significant blood clot. Furthermore, platelet aggregation and adhesion were weakened. Considering the information obtained from this model, the authors presumed that anemic inflammation, brought about by HAMP-mediated iron sequestration in the liver, macrophages and spleen, might be viewed as a functional iron deficiency (ID) and affected patients should be treated as thrombosis high risk patients especially COVID-19 patients[166]. Taken together, the diverse activities of CQ and HCQ as an anti-inflammatory, antithrombtic and antiviral medication might also be connected to their effects on iron homeostasis, both systemically and locally. Noticeably, a different regular treatment, routinely utilized in the management of COVID-19 patients with predominantly increased levels of heparin. Similar to CQ and HCQ, heparin is a universal drug, as characterized by Jecko Thachil[167], with potential activity such as anti-inflammatory, anti-viral and anti-coagulant medication. It is noteworthy that CQ or HCQ as well as heparin turned out to control iron metabolism: HAMP expression in human macrophages, FPN1 expression in the plasma-membrane and advance iron export, leading to cellular starvation of iron, antithrombotic activities are part of all the remarkable properties of this drug[168]. These findings recommend a possible function of iron in SARS-CoV-2 infection that needs to be investigated in future for clinical studies, it should be equally considered as a possible target for COVID-19 treatment as proposed for other infectious diseases in humans in the past[126,153,154].

Safety Considerations

CQ and HCQ have a well-considered profile. This medication has been utilized for over 50 years' in the treatment of malaria showing the safety of the administration of chloroquine to individuals. The utilization of CQ and HCQ in the treatment of rheumatic diseases and for antimalarial prevention indicated that there is a low rate of unfavorable occurrences during constant administration of this medication for a couple of years. In these circumstances, macular retinopathy is the most serious side effect, which occurs as a result of the cumulative dose instead of daily dose, while permanent injury might be prevented with normal visual examination during management[51,169,170]. A new report[171] gave an encouraging conclusion on what to expect during high dosage authorizing results on the safety of a high dosage of the medication (about 500 mg of chloroquine base every day) even at gestation period. We presume that CQ and HCQ administration offers controlled and limited toxicity and may consequently bring about a generally safe/advantage balance when it is utilized in life frightening conditions. Consequently, we resolve to examine the suitability of this old medication in

the management of dual infectious diseases presenting a danger to global health in the period of globalization, AIDS, as well as severe acute respiratory condition (SARS). These illnesses are both brought about by enveloped RNA viruses, and offer some clinical signs that are probably going to be interfered with by immune responses of the host[11]. CQ and HCQ are startlingly poisonous when overdose is administered, showing a few signs similar to cyclic anti-depressing toxicity. Persistent or spontaneous overdose prompts quick initiation of central nervous system toxicity (seizures and unconsciousness), cardiac breakdown (with inhibition of the heart sodium and potassium channels bringing about QRS elevations and QT interval extension, respectively) and hypokalemia resulting from intracellular flunctuations[172]. Management of overdose strongly requires incorporation of brief therapy of activated charcoal, intravenous benzodiazepines and vasopressors fluctuating, sodium bicarbonate or hypertonic saline for substantial QRS amplification and interrelated arrhythmias, as well as cautious administration of hypokalemia to avoid overcorrection. Strict monitoring from a toxin control center is recommended in every cases.

Could Consumption of These Medicines Possibly Exacerbate COVID-19 Infection?

In spite of the positivity associated with ability of chloroquine and/or hydroxychloroquine in the management of COVID-19, few considerations have been given to the likelihood that the medications may adversely affect the development of infection. For instance, a few proposed that inhibition of T-partner cell expansion and interleukin-2 formation or reaction may coincidentally increase the inflammatory reaction in infected patients, perversely affecting the health of the patients[173]. The likelihood that these medications may present unfavorably effect points out the need for high-quality randomized controlled clinical trials even with an emerging pandemic[107]. The immunomodulatory effects of CQ and HCQ are basic to a subset of patients with rheumatoid joint pain, ankylosing spondylitis, systemic lupus erythematosus, sarcoidosis and other chronic infections. Increased treatments based on theory of their functions interactions or therapy in SARS-CoV-2 infection by patients having chronic disorders should be critically examined. In this light, two drug manufacturing companies have declared their plans to increase hydroxychloroquine production fully anticipating this necessity[107].

Conclusions

COVID-19, an infectious disease caused by the novel coronavirus advances through different means to induce infection and its spread[174]. The outbreak of COVID-19 has imposed threat to global health and economy characterized by its increased incidence and mortality globally[175]. The main proponents of drug repurposing in the proposed management of SARS-CoV-2 infection are CQ and HCQ. Hence, the functional role of CQ and HCQ was analyzed providing possible mechanistic insight into the role of iron on viral infection, iron starvation and its downstream cellular pathways involving hepcidin and proinflammatory cytokines. Overall, the possible mode of action of CQ and HCQ in the management of COVID-19 infection is exhibited via its anti-viral, anti-inflammatory and anti-thrombotic activities which could be considered in viral diseases linked to inflammation or potentially immune initiation. Cytokine inhibition by CQ or HCQ, through their action on iron homeostasis, could reduce inflammation, reestablishing a reasonable basis for iron homeostasis and erythropoiesis. CQ or HCQ have their action on cellular immunity through iron starvation, considering the natural and adaptive immune responses against viral infections.

Conflicts of Interest

The authors declare no conflicts of interest.

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