Research Article

Reconsidering Chloroquine and Hydroxychloroquine in Treatment of COVID-19: Lessons Learnt

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Abstract

The pandemic of Coronavirus Disease 2019 (COVID-19) caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been difficult to manage globally due to the absence of proven effective therapeutic options. While there are numerous studies that are currently underway to develop new medications or vaccines to cure the deadly disease and to prevent its spread, the time needed to accomplish this is long. In view of the urgency of this situation, there is a need to also consider the possibility of identifying a cure using the existing medications with known safety and toxicity profiles. So there has been a renewed interest in studying the therapeutic effect of available drugs with anti-viral and anti-inflammatory activity, like Hydroxychloroquine (HCQ) and Chloroquine (CQ), which have been used successfully for a long time to treat malaria and rheumatic disorders, on the pathogenesis of COVID-19, with the hope of using these well studied drugs as potential treatments of this disease. A literature review was conducted using Pubmed/MEDLINE, Cochrane, EMBASE electronic databases and clinical medicine preprint repository medRxiv, along with review of clinical trial data for use of CQ and HCQ in treatment of COVID-19 to compile the results of clinical and pre-clinical studies reported. This review is intended to provide a comprehensive summary of the currently available evidence-based information regarding the pharmacological and pharmacokinetic properties of CQ and HCQ and their safety and effectiveness in reducing the severe inflammation associated with coronavirus infections, as well as what we have learnt about their adverse effects when used as an anti-viral drug, in order to provide an informed understanding of their current clinical uses and potential for their safe and effective use in future treatment.

Keywords: COVID-19 treatments, Hydroxychloroquine, Chloroquine, Coronavirus disease

Introduction

On the 11 March 2020, the World Health Organization (WHO) pronounced COVID-19 disease to be a pandemic, causing severe and frequently deadly, respiratory ailment in different regions globally. So far, there are no medications that has been demonstrated to be effective against this disease. It is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which is one of the several coronaviruses, which have crossed the species barrier into humans and bring about various forms of respiratory illness in numerous geographic locations. Novel medications and/or immunizations will take time to be developed and disseminated to patients. Therefore, there has been growing interest in the reconsidering the use of existing medications, such as Chloroquine (CQ) and Hydroxychloroquine (HCQ), as potential treatments of this disease.

The newly emerging SARS-CoV-2, the causative organism for COVID-19, has approximately 200 published virus sequences, whereas other lineages, including Middle East Respiratory Syndrome-Related Coronavirus (MERS-CoV), has over 500 viral sequences. After binding the receptor, the virus undergoes cleavage by a nearby host protease, releasing the spike fusion peptide, facilitating virus entry. [1] Known host receptors for betacoronaviruses include Angiotensin-Converting Enzyme 2 (ACE2) for SARS-CoV and dipeptidyl peptidase-4 9DPP4) for MERS-CoV. [2,3] But recent studies have demonstrated how different coronavirus lineages can be divided into clades based on the Receptor-Binding Domain (RBD) region of the spike protein mediating the interaction with the host-cell receptor, can recombine to enter human cells. This group also confirmed that human ACE-2 is the receptor for the recently emerging SARS-CoV-2. [4]

Research will be needed to determine structural characteristics of SARS-CoV-2 that underlie pathogenetic mechanisms. The data so far available seem to indicate that the viral infection can

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produce an excessive immune reaction in the host. In some cases, a reaction takes place which is collectively called a 'cytokine storm', leading to extensive tissue damage. The protagonist of this storm is Interleukin 6 (IL-6). IL-6 is produced by activated leukocytes and acts on many cells and tissues. It can promote the differentiation of B lymphocytes, promotes the growth of some cell types, and inhibits the growth of others. It also stimulates the production of acute phase proteinsand plays an important role in thermoregulation, in bone maintenance, and in the functionality of the central nervoussystem. Although the main role played by IL-6 is pro-inflammatory, it can also have anti-inflammatory effects. In turn, IL-6 increases during inflammatory diseases, infections, autoimmune disorders, cardiovascular diseases and some types of cancer. It is also implicated into the pathogenesis of the Cytokine Release Syndrome (CRS) that is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction. [5]

The aminoquinolines CQ and HCQ are widely used in the treatment of malaria and rheumatic diseases, and they have been suggested as effective treatments for coronavirus disease 2019 (COVID-19) due to their anti- inflammatory and antiviral effects. [6-9] In the United States, the Food and Drug Administration (FDA) issued an Emergency Use Authorization on March 30, 2020, that allowed the use of these drugs in patients with COVID-19 who were not enrolled in clinical trials. Guidelines suggested that these drugs be administered to hospitalized patients who had evidence of pneumonia [10], and to date, they have been used in many thousands of patients with acute COVID-19 around the world. However, to date, there have been no robust clinical trials that haveshown efficacy of these agents for this illness, and the data that are available come from small studies that have either been uncontrolled or underpowered to detect meaningful clinical effects. HCQ is a more soluble and less toxic metabolite of CQ. Recent work suggests that HCQ has more potent antiviral properties than CQ, and it causes fewer side effects and is therefore considered safer. [11] Based on the data reported in these studies, HCQ was suggested as treatment for hospitalized patients with COVID-19 and respiratory difficulty, as indicated by a low resting oxygen saturation, during the period of hospitalization. The original report of HCQ as a treatment for COVID-19 described 26 patients who had been treated in an open-label, single-group study that involved contemporaneous, but nonrandomized controls in hospitals in France. [12]

Patients were treated with HCQ at a dose of 200 mg three times daily for 10 days. Data from this study were reported as showing the effectiveness of HCQ in reducing the viral burden in treated patients (65.0% clearance by day 5, vs. 18.8% clearance by day 5 in untreated patients). However, data from 6 patients who received HCQ were excluded from the analysis because of clinical worsening or loss to follow-up, which makes it difficult to interpret the findings. Very recently, an observational study on the association between HCQ use and respiratory failure in a substantial number of patients with COVID-19 in New York City reported that HCQ administration was not associated with either a greatly lowered or an increased risk of the composite end point of intubation or death. [13] Therefore, better designed randomized, controlled trials of HCQ in patients with COVID-19 are needed.

This review article is intended to be a comprehensive source of evidence- based information regarding the pharmacological and pharmacokinetic properties of CQ and HCQ as a basis for understanding their current clinical uses and potential for their safe and effective use in COVID-19 patients.

Overview of Chloroquine and Hydroxychloroquine

Clinical Use

HCQ and CQ have been utilized primarily in the prevention and treatment of malaria and in treatment of inflammatory rheumatic diseases such as Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). Both are on the World Health Organization list of essential medications [14], a list which includes medications considered the most efficacious, safe, and cost-effective for basic health-care systems. HCQ, the hydroxylated derivative of CQ, has lower incidence of overall toxicity and is widely regarded as a safer option. [15]

These medications have a myriad of effects at the cellular level, and the relative impact that of each of these mechanisms play in the treatment of disease remains poorly understood. Of primary importance, the immunomodulatory and anti-inflammatory effects are key in reducing organ damage and preventing flares in rheumatic diseases. [16] As antimalarial drugs, these medications accumulate in the parasitic digestive vacuoles and interfere with hemoglobin breakdown and heme polymerization leading to selective toxicity. [17,18] Interestingly, these medications also appear to confer beneficial metabolic effects such as improvements in lipid profile and reduction in hyperglycemia. [19–21] HCQ and CQ are recognized as potential antiviral drugs based on in vitro studies demonstrating interfere with endosome-mediated viral entry and viral replication of enveloped viruses [22], included coronaviruses. [23] As cost-effective medications with a wide spectrum of cellular mechanisms, there is immense interest in repurposing these medications for other conditions. [22]

Dosing

The precise dosing of HCQ and CQ is dependent on the indication. Dose response relationship are not clearly established due to complex pharmacokinetic profiles. [24] These medications are primarily given by the oral route with rapid absorption in the upper gastrointestinal tract and bioavailability of around 0.7-0.8. [25] For malaria prophylaxis, around 500 mg of CQ phosphate or 400 mg of HCQ sulfate is recommended weekly in adults. In acute treatment of malaria, loading doses leading doses are given on the first day with 3 days of total treatment (800 mg, then 400 mg at 6 hours, 24 hours, and 48 hours or 1000 mg, then 500 mg at 6 hours, 24 hours, and 48 hours for HCQ sulfate and CQ phosphate, respectively). For chronic treatment of rheumatoid disorders such as SLE, a daily dose not exceeding 400 mg-500 mg of HCQ sulfate is often recommended to limit serious adverse effects such as retinopathy.

Similarly, the US FDA suggested a dose of 800 mg of HCQ sulfate on day one, followed by 400mg daily for four to seven days of total treatment in adults and adolescents weighing at least 50 kg. [26] Alternatively, another dosing paradigm utilized 200 mg of HCQ sulfate three times per day for ten days, with or without azithromycin. [11,12]

Despite in vitro data demonstrating inhibitory activity against SARS-CoV-2 [11,27], it remains unknown whether these medications translate to improved clinical outcomes and the ideal dosing scheme to achieve those outcomes.

Pharmacokinetics

HCQ and CQ are structurally classified as 4-aminoquinolines, containing an aromatic ring and a basic side chain, with HCQ being the hydroxylated derivate of CQ. The basic side chain is essential for accumulation of these agents in acidic vesicles such as lysosomes though ion trap accumulation, which is critical for their mechanism of action. [28] CQ and HCQ are given as enantiomers, and evidence suggests stereoselectivity for distribution and metabolism and potentially efficacy and safety based on these isomers. For example, (S)-(+)-HCQ has a higher bioavailability but shorter half-life, and may offer reduced incidence of certain adverse effects such as retinopathy. [29]

Following absorption from the upper gastrointestinal tract, HCQ and CQ have a long half-life of approximately 40 days-60 days [30], due to a large volume of distribution and accumulation in intracellular compartments. [31] Importantly, there is a wide variability of serum concentrations among patients, with one study indicating that levels varied eleven-fold among patients with RA. [25] The drugs are about 30%-50% bound to plasma proteins such as albumin and alpha glycoprotein and display prominent tissue binding, which can be beneficial for treatment related to a specific tissue, but problematic for tissue-dependent adverse effects. For example, binding of HCQ/ CQ to melanin contributes to build-up in the eye and retinopathy. [25] Hepatic metabolism and renal elimination are the primary mechanisms for drug clearance. Dealkylation by various CYP isoforms including CYP3A4, CYP2C3, CYP2D6, and CYP1A2 leads to the formation of several metabolites, some of which are active. [32] Based on the wide variability in serum concentrations and potential for toxicity, PKPB models have been proposed to utilize patient characteristics to predict exposure in various compartments and reduce the risk of toxicity. [33] Thus, these models may serve as promising clinic tools for utilization of HCQ/CQ in various patient populations.

Adverse Effects

Although considered to have a generally favorable safety profile, there are serious adverse effect associated with HCQ and CQ, with HCQ associated with a lower incidence of adverse effects. [15] Common adverse effects include gastrointestinal effects such as abdominal pain, vomiting, and diarrhea which may be related to abdominal muscular effects, and dermatologic allergic reactions, often in the form of pruritic maculopapular lesions, in up to ten percent of patients. [34] The primary concerns related to the development of severe adverse effects include cardiotoxicity and retinopathy. The long half-lives of CQ and HCQ, which may be detected for years after discontinuation due to sequestering and slow release, may pose a particular challenge when considering serious adverse effects. [35]

HCQ and CQ accumulate in the eye due to binding to melanin within the retinal pigment endothelium. Here, the medications inhibit lysosomal activity leading to a reduction in phagocytosis of photoreceptor segments and intraretinal accumulation. [36] Over time, atrophy of the retinal pigment epithelium may occur leading to the potential for permanent loss of vision. [37] Additional manifestations of accumulation in the eye include corneal deposits due to deposition in the corneal basal epithelial layer; however, corneal deposits are reversible and do not result in optic damage. [38] Some risk factors for increased risk of retinopathy include higher doses (particularly>6.5 mg/kg for HCQ or >3 mg/kg for CQ), duration of use exceeding 5 years, age >60 years, or concomitant renal, liver, or retinal disease. [37] Duration of therapy may be the best predictor of risk of toxicity, with one study finding an overall prevalence of around 7.5%in patients taking HCQ for at least 5 years which increases to nearly 20% in those taking the medication for more than twenty years. [39]

Cardiotoxicity has been described as a rare but serious complication related to the use of CQ or HCQ, and prevalence of cardiotoxicity is thought to be much less compared to retinopathy. [40] CQ is associated with a higher risk of cardiotoxicity than HCQ, but cases associated with HCQ are increasingly reported, likely due to the prevalence of use. Types of cardiotoxicity reported include cardiomyopathy which may be classified as restricted or dilated, and conduction abnormalities including corrected QT (QTc) prolongation-induced arrhythmias. [41]

Development of QTc prolongation is of particular concern related to the treatment of COVID-19, and has been reported more frequently in this population, especially when HCQ is given in combination with azithromycin. [42] The mechanism for QTc prolongation is related to structural similarities with antiarrhythmic medications and potential for inhibition of potassium channels, which prolongs the cardiac action potential. [28] It is suggested that COVID-19 patients may be at higher risk of QTc prolongation and the associated life threatening arrythmia, Torsades *de* Pointes (TdP), due to a relatively high prevalence of cardiovascular disease in the hospitalized population, presence of critical illness, and the use of concurrent medications like azithromycin that can also prolongthe QTc interval [42] Although data collection in this population is still in an early stage, a recent report found that around 20% of hospitalized COVID-19 patients experienced a QTc prolongation of ≥ 500 ms, and one patient out of 90 experienced TdP. Thus, increased vigilance and monitoring is suggested related to the risk of arrythmias in hospitalized patients with COVID-19.

Drug Interactions

Although drug interactions with CQ and HCQ are not exceedingly common, some clinically important interactions have been reported. Various mechanisms can contribute to drug interactions involving CQ and HCQ include CYP interactions, alterations in absorption, and mechanism-based interactions.

CQ and HCQ reduce the serum concentrations of methotrexate, although the mechanism is not well-understood and this interaction does not appear to lead to clinically important alterations in efficacy. [43,44] It is suggested that the interaction is related to alterations in gastrointestinal absorption, either by local alterations in pH or alterations in gastric emptying. [25,43] Conversely, medications that increase gastrointestinal pH, such as proton pump inhibitors, might

interfere with theabsorption of CQ and HCQ. [45] CYP based interactions have also been reported. HCQ reduces the metabolism of certain beta blockers such as metoprolol, possibly by competitive inhibition of CYP2D6 [46], although the clinical impact of this is uncertain. Another potential concern is additive hypoglycemia when added on to antidiabetic agents, possibly related to an increase in serum insulin levels. [47]

Perhaps the most concerning drug interactions are those related to enhanced probability of serious adverse effects including retinopathy and QTc prolongation. Co-administration of tamoxifen is recommended to be limited to six months because of increased risk of retinopathy due to synergistic effects in retinal epithelial cells. [48] An increased risk of arrythmia is a concern with co-administration of other QTc prolonging medications, including Class III antiarrhythmics and quinidine, some antipsychotics and antidepressants, and antibiotics including fluroquinolones and macrolides. [49] As previously mentioned, co-administration of HCQ and azithromycin has posed a particular concern among hospitalized patients with COVID-19. [42] Thus, awareness of these potential interactions is essential when weighing the risks and benefits of therapy.

Potential Mechanisms of Action in the Treatment of COVID-19

In recent pandemic context based on several studies and discussion for CQ and HCQ are become a hot topic across the world [50] Several other possibilities to control or prevent this emerging infection, including vaccines, monoclonal antibodies, oligonucleotide-based therapies, peptides, interferon therapies and small-molecule drugs are also being studied. [51] Although HCQ and CQ are well-known Disease-Modifying Antirheumatic Drug (DMARDs) but it has been used for decades as the primary and most successful treatment option against malaria. Significant controversy has been raised regarding the exact mechanisms of action of these molecules for the treatment of SARS-CoV-2. [53–55]

CQ/HCQ has ability to accumulate in acidic compartments such as lysosomes and inflamed (acidic) tissues, inhibit autophagy, and inhibit immune activation of different cell types, as well as its role in reticence of cytokine production and modulation CD154 expression on T cells [56,57] (Figure 1). Previous clinical studies (Table 1) indicate that large volume distribution and long half-lives of these drugs can explain some of their clinical characteristics, such as their slow onset of action and prolonged effects after drug discontinuation. [58] At the cellular level, several direct and indirect mechanisms have been described related to the immunomodulatory role of CQ/HCQ in Covid 19. [59-84]

Alterations in ACE2

Previous studies suggest that cellular entry of SARS-CoV-2 through the host protein Angiotensin-Converting Enzyme-2 (ACE2, EC 3.4.17.23) also acts as a coreceptor to gain intracellular entry into the lungs and brain [4,85,86]. ACE2 is a membrane-bound peptidase with the majority of the NH2-terminal peptide domain including the catalytic site oriented extracellularly. [87] ACE2 is expressed in essentially all tissues, with greatest activity in the ileum and kidney followed by adipose tissue, heart, brain stem, lung, vasculature, stomach, liver, and nasal and oral mucosa based on activity data in the mouse that generally parallel ACE2 mRNA levels in humans. [88,89] ACE2 belongs to the Renin-Angiotensin-Aldosterone System (RAAS), which plays important roles in regulating blood pressure and body fluid, contributing to the pathophysiology of hypertension and cardiovascular/renal diseases by maintaining homeostasis of blood pressure, electrolyte balance and inflammatory responses. [90] Attenuation of ACE2 catalytic function alters RAAS system activity, resulting in enhanced inflammation and vascular permeabilityobserved in the pathogenesis of inflammatory lung disease. [91]

Table 1: Effective Combination Therapeutic Options with HCQ for the Treatment of SARS-CoV-2 Infection.

Major Organ/Systems	Major adverse effects	Estimated frequency	Evidence against for use	Evidence beneficial effect of use
Cardiovascular	Conduction disorders cardiomyopathy	occasionally	YES [41,61]	YES (Sharma et al., 2016,Hartman et al., 2017)
Endocrine and Metabolism	Hypoglycemia	less common	YES [64]	YES [20]
Hematologic	Hemolysis, leucopenia, aplastic anemia	less common	YES [65]	No Evidence
Hepatic/Biliary/Pancreatic	Gastrointestinal discomfort	most common	YES [66]	No Evidence
Neurologic	Neuromyopathy, seizures, psychosis, photosensitivity	several reports suggested more common	YES [67]	No Evidence
Ophthalmologic	Photosensitivity, keratopathy, retinopathy	low incidence, higher dose and/or prolonged periods	YES [68]	No Evidence
Psychiatric	Psychomotor agitation, psychosis and suicidal tendencies	less common	YES [69,70]	No Evidence
Skin	Allergic contact dermatitis, Rash	occasional	YES [71]	No Evidence
Ears	Ototoxicity	rare	YES [72,73]	No Evidence
Musculoskeletal system	Myopathy	occasional	YES [74,75]	YES [76]
Urinary tract system	Impaired renal function	occasional	YES [77,78]	YES [79]
Pregnancy	CNS damage, retinal hemorrhages and abnormal retinalpigmentation to the fetus.	rare	No Evidence	YES [80,81]
Carcinogenesis and Mutagenesis	Produce glioblastoma	rare	YES [82]	YES [83,84]

The entry of the virus in the cells is mediated by spike (S) glycoprotein; (Fig-1) particularly; the spike 1 (S1) surface unit allows the attachment of the virus to cellular receptors. [92] To allow the entry of the viral particles, the S protein is cleaved by cellular proteases at the S1/S2 and the S20 site. Then, the viral capsid is fused with the cellular membrane driven by the S2 subunit. [92] SARS-CoV 2 entrance is mediated by Angiotensin-Converting Enzyme 2 (ACE2), and the serine protease TMPRSS2 is responsible for the S protein cleavage. [92,93]

ACE 2 receptor based key treatment strategy for SARS-CoV-2 cellular entry:

1. The in-silico analysis of the sequences for Receptor Binding Motif (RBM) revealed that some antibodies developed against human ACE2 can be useful for blockage of SARS-CoV-2 infections. [94,95]
2. Another promising approach to block viral entry includes the use of natural neutralizing antibodies from convalescent sera and engineered antibodies. Engineered antibodies or neutralizing fragments can be in various formats, such as soluble receptor-binding domain (based on SAR-S protein) that would occupy ACE2 and prevent access to SARS-CoV-2; antibodies or single chain variable fragment that would bind to ACE2 and inhibit entrance to SARS-CoV-2. [96]
3. Recent report highlights Emodin (a naturally occurring anthraquinone] and promazine [phenothiazine class of anti-psychotics) have been shown to interrupt the binding of S protein with ACE2. [97] Further, drug- repurposing strategies have suggested possible small molecule drugs that may bind to S-protein to disrupt S protein-ACE2 interaction. [97–99]

CQ and HCQ are currently two of the most widely tested molecules for the treatment of COVID-19. [27,100] Although evidence of CQ and HCQ is limited (based on the *in vivo* experimental data and only two small human trials), but studies on HCQ with NH4Cl cell culture conditions showed that the cell surface expression of under-glycosylated ACE2 and its poor affinity to SARS-CoV 2 spike protein may be the primary mechanism by which infection is prevented by drug pretreatment of cells prior to infection. [12,101] On the other

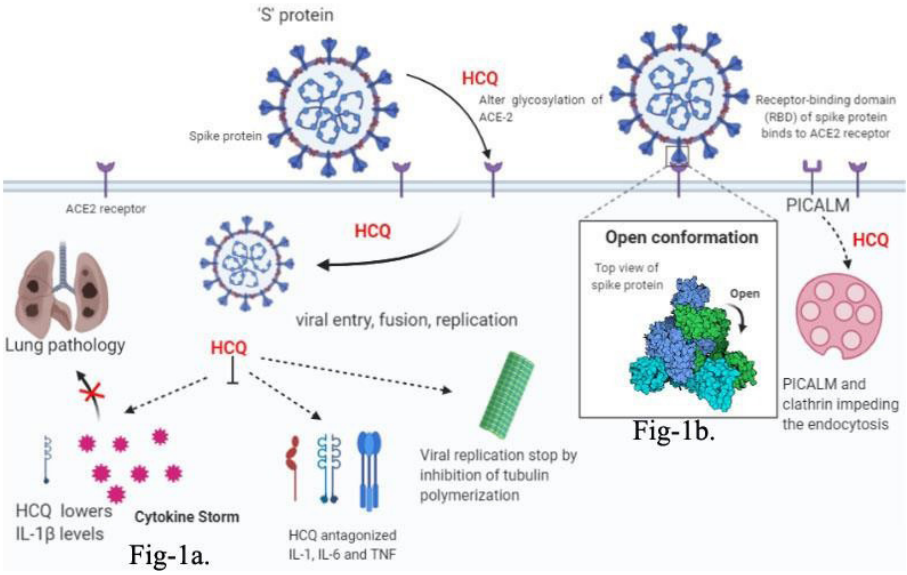

Figure 1: Pathogenesis of COVID-19 infection. 1a. Multiple therapeutic effects of HCQ during COVID-19 infection including inhibition of viral S protein attachment to host cell angiotensin converting enzyme 2 (ACE2) and PICALM mediated entry, effect on host cytokine storm, inhibition of viral replication 1b. Viral attachment and conformational change of ACE2 receptor and role of HCQ as a autophagy related machinery inhibitors.

Table 2: Effective Combination Therapeutic Options with HCQ for the Treatment of SARS-CoV-2 Infection.

Product Type and Candidate	Act as on	Combination with	Clinical Feedback for COVID 19 Treatment	Reference
Antibiotics [Azithromycin Teicoplanin]	Broad-spectrum Macrolide antibiotic Glycopeptide antibiotic	HCQ, CQ	No well-controlled, prospective, randomized clinical evidence	[12,102]
Zinc	· Zinc ions accumulated in the lysosomes where in presence of HCQ it impair of function of lysosomal enzymes · Inhibits RNA polymerase		No such documented trail so far but evidence based study suggest zinc ionophore increase the potency of HCQ while in combination	[103–105]
Ivermectin	Anti-parasitic drug, specific inhibitor of importin-α/β- mediated nuclear import		No documented trail so far	[106]
Anakinra	Recombinant human IL-1 receptor antagonist		Long-term Anakinra found immunosuppressive and/or immunomodulatory agents	[107]
Prednisone			Prednisone with HCQ	[107]
Metformin	anti-diabetics		Adverse drug reactions noted	[12, 108]

hand, rapid elevation of endosomal pH and abrogation of virus-endosome fusion may be the primary mechanism by which virus infection is prevented under post-treatment conditions. [27]

Considering the potentially favorable benefit-risk balance of all these possibilities, Table 2 represent potential therapeutic combinations with CQ/HCQ. It is important to characterize the RAAS phenotype before considering any treatment based on this principle, since RAAS has both an immediate role in blood pressure and fluid balance regulation and a longer-term impact on chronic oxidative stress, inflammation and fibrosis. [90]

Lysosomal alterations

In the context of the COVID-19 pandemic, scientists around the world are straggling to identify most effective drugscapable of preventing or treating this infection. Following the binding ACE2, SARS-CoV-2 undergoes internalization and trafficking into the endosomes and eventually, the lysosomes, which constitute key intracellular players in viral uncoating and fusion.

However, after binding to ACE2 and prior to internalization, the S protein is subject to enzymatic modification by the Transmembrane Serine Protease 2 (TMPRSS2), a membrane protein residing in the vicinity of ACE2. [109,110] Specifically, TMPRSS2 induces a cleavage mediated conformational change in the S protein, as well as in ACE2, which allows the host cell membrane to invaginate, which is a crucial step in initiating viral endocytosis. [109]

Following viral endocytosis now this viral S protein undergone a series of enzymatic cleavages and modifications bycathepsin L, and to a lesser degree by cathepsin B, lysosomal cysteine proteases encounter the virus in the endo- lysosomes. [111] These steps play a major role for viral membrane fusion and subsequent release of its RNA genome into host cytoplasm [111] In fact, the latter step has been shown to be highly pH-dependent, with fusion only occurring after reaching highly acidic compartments [i.e., the lysosomes], which possess the highest cathepsin L activity. [112] A recent study by Holliday et al., showed Bafilomycin is one of the drugs identified which inhibits ATP6AP1, an ATPase inhibitor. This enzyme was "fished" by using the Nsp6 protein of the SARSCoV2. [113]

One proposed mechanism of CQ and HCQ is by increasing the pH of the lysosome and impairing viral fusion and assembly. If this is true, it would be useful to use during the early phase of infection and as a prophylactic agent. Bafilomycin may enhance this activity. Several studies indicate CQ and HCQ can inhibit lysosomal fusion with endosomes, as well as inhibiting the activity of its enzymes. [59,114] After its entry into cells, CQ becomes protonated and entrapped within the lysosomes where it can induce similar aberrations in lysosomal function, primarily by elevating lysosomal pH and inducing partial lysosomal membrane permeabilization that interferes with intracellular viral transport and fusion. [115]

Finally, another study reveals that lysosomal proteases are key mediators of coronavirus tropism and infection in bats, the natural reservoirs of the virus. [116] This is another reason to highlight the importance of considering the lysosome as a potential target of therapeutic intervention against COVID-19.

Reduction in inflammatory cytokines

Raising cytokine levels is the most serious outcome for COVID-19 infection. [98] This is also known as Macrophage Activation cytokine (MAS),storm, or hypercytokinaemia. Due to the lack of definite therapy and non-existent herd immunity, anti-cytokine therapy, especially anti-IL-6 and others like IL-1 antagonism have been proposed for mitigating against the hyper-inflammatory syndrome. [117,118] Covid-19 infection in the lower airways initiates an uncontrolled immune-mediated inflammatory response. T lymphocytes are the main target cells in Severe Acute Respiratory Syndrome (SARS) due to COVID-19, triggering cytokine storm with

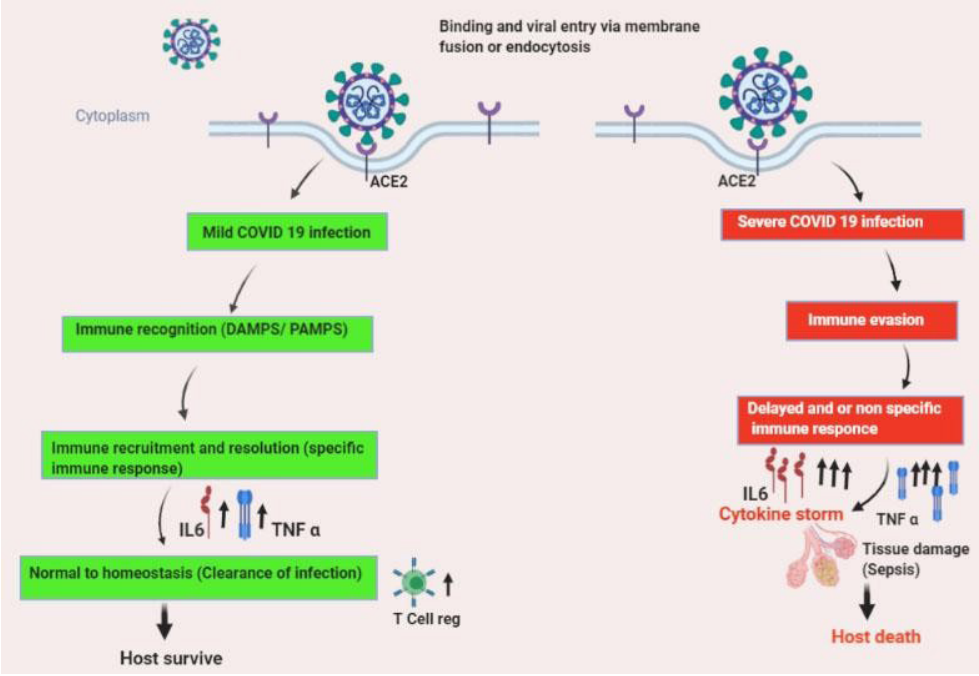

Figure 2: Comparison of host pathophysiology against mild to severe SARS-CoV-2 infection.

Table 3: Common Strategy of Therapeutic Targeting Against Cytokine Storm.

Therapeutic agents	Mechanism of action	Drug adverse events	Cost benefit	Reference
Corticosteroids 10197.88 [approximate]	Immunomodulatory role	· Increased long-term mortality · secondary infection	Very expensive	[122]
CQ and HCQ Less than $ 50 (approximate)	·Reduce cytokines production [TNF, IFN, and IL-6] · Reduce T-cell proliferation · Reduce TLR activation · Reduce platelet aggregation	Adverse events other than GI complications [i.e., neurologic, ophthalmic, genitourinary, allergic, cardiovascular, musculoskeletal, infectious disease, psychological, and were dose dependenthematologic systems]	Less expensive	[60, 123, 124]
Monoclonal antibody (Tocilizumab) $500 [approximate]	· Blocks IL-6 · Specifically to sIL-6R and mIL-6R, and block signal transduction	Increased risk of opportunistic infections	Less expensive	[118, 125-127]
Other immunomodulatory agents Urinary trypsin inhibitor [also called Ulinastatin or UTI] $1197.09 [approximate]	· Inhibitor with anti- inflammatory properties [including inhibition of IL-6]	No such adverse effects were seen	Expensive	[128, 129]

subsequent exhaustion of immune response. [119,120] Based on pathophysiology and clinical manifestations of Covid-19, the justification for searching specific antivirals along with immune-modulating drugs is admissible. According to the US National Library of Medicine Clinical Trials registry (https://clinicaltrials.gov/) it is clear that anti-rheumatic drugs are being investigated COVID-19. In this review we have tried to summarize COVID-19 associated pulmonary immunopathology and the advantage or disadvantages of IL-6 antagonism is patients with ARDS that has consequences of other anti-cytokine strategies including IL-1, IL-18 or IFNγ antagonism (Fig-2). Therefore, we focused on IL-6 and its relationship to the COVID-19 MAS-like pathology but several other relevant cytokines including Il-18, IFNγ, and the JAK1 pathway critically control macrophage function including IL-6 production during MAS states. [121]

Selection of the best possible therapy for patient management has a significant role in this pandemic situation. Several evidence-based options were summarized in (Table 3) for the clinical management of cytokine storm condition with a cost benefit prospective.

*Very expensive: more than 5000$, Expensive: > 1000$ Less expensive: ≤500$, Costs may vary in different settings because of negotiated procurement discounts and the variability of doses.

Synergistic therapies in the treatment of covid-19

A few combinations with HCQ/CQ are currently being explored for potential synergistic activity in the treatment of COVID-19. Particularly, the combination of azithromycin and HCQ has been widely utilized. Although there is promising in vitro data for antiviral activity against COVID-19, the published reports for clinical efficacy have been mixed and randomized clinical trials are pending. Zinc is also proposed to provide synergistic antiviral effects when combined with HCQ, although very little data exists for potential antiviral efficacy of the combination. Several other agents are being explored in combination with HCQ in clinical trials based on complementary mechanisms of action. There is great interest in the possibility of combination treatments providing higher efficacy against COVID- 19 compared to monotherapy.

Azithromycin

Azithromycin is a macrolide antibiotic with a relatively broad spectrum against gram positive and gram-negative bacteria. It penetrates lung tissue well [130] and thus is often used in bacterial respiratory infections and in prevention of chronic obstructive pulmonary disease exacerbations. In addition to its antibacterial activity, azithromycin and other macrolides are also reported to have antiviral activity in vitro. [131–133] Azithromycin may also reduce inflammation by reducing arachidonic acid release leading to a reduction in the synthesis of eicosanoids. [134,135] Based on the antiviral and anti-inflammatory properties of azithromycin, it is suggested that it could be utilized in the treatment of COVID-19.

HCQ and azithromycin each have antiviral effects in vitro, but they may also display synergy as antiviral agents. Since both azithromycin and HCQ accumulate in lysosomes as weak bases, it has been suggested they may provide complementary inhibition of viral replication when administered concurrently. [102] Importantly, a recent report demonstrated a synergistic effect of the two drugs in significantly inhibiting viral replication of SARS-CoV-2 utilizing concentrations which are attainable in human lungs. [136] Follow-up studies are needed to validate these results and better understand the in vitro effects of combination therapy. As previously mentioned, there are safety concerns with this combination related to prolongation of the QTc interval, which can lead to life threatingarrythmias.

In a small, non-randomized, single arm study from France, in COVID-19 patients 100% (6/6 patients) patients who received the combination of azithromycin and HCQ tested negative compared to 57% (8/14) patients receiving HCQ alone and only 12.5% (2/16) of untreated patients [12] indicating that HCQ seemed to enhance viral elimination with azithromycin providing additional efficacy. The same group of investigators subsequently published another non-randomized study in which 97.5% of mildly infected patients receiving the combination of HCQ and azithromycin had negative respiratory cultures at day 5. [137] However, another group in France reported outcomes for combination treatment of azithromycin and HCQ in a small group of patients and found that 8/10 still tested positive for COVID-19 at days 5-6 after treatment initiation. [138] Thus, the clinical efficacy of combination therapy is unknown. It is will be essential to determine whether the combination of HCQ and azithromycin is safe and effective in the treatment of COVID-19 in randomized, controlled trials.

Zinc

Zinc is an essential trace element and a widely available supplement which is recognized for its ability to reduce the duration of the common cold, often caused by rhinovirus. [139] Zinc inhibits the replication of viruses including Coronaviruses. [140] Although the precise mechanism is unclear, zinc seems to inhibit the activity of RNA-dependent RNA polymerase in vitro which is essential for viral replication. [140,141] Importantly, CQ enhances the uptake of zinc into cells based on its ability to bind to and transport zinc. [104] Therefore, CQ and HCQ have been referred to as zinc ionophores. [142] Importantly, the activity of zinc in the impairment of RNA-dependent RNA polymerase is greatly enhanced through the utilization of Zinc-binding substances that improve intracellular uptake. [140] Additionally, zinc may improve the sequestration of CQ and HCQ into acidic organelles such as lysosomes and enhance the efficacy of these medications. [104] Thus, it has been proposed that zinc and CQ/HCQ possess synergic activity, and that the combination may be more efficacious than CQ/HCQ alone in the treatment of COVID-19. [142,143] Although the antiviral properties of the agents are established in vitro, whether the combination displays synergy in antiviral effects in pulmonary tissue in individuals infected with COVID-19 is unknown. Currently, most ongoing clinical trials have not added an arm of HCQ plus zinc versus HCQ alone in the treatment of COVID-19. There are, however, some clinical trials looking at the combination of HCQ with zinc and vitamins, such as vitamin C and D, in prophylaxis against COVID-19 in high risk individuals. [144,145]

Other combinations

Other combination therapies with HCQ have been proposed in the treatment of COVID-19 and are undergoing clinical trials. One clinical trial is underway in Germany to evaluate the safety and efficacy of HCQ and Camostat combination therapy versus HCQ monotherapy in patients with a moderate COVID-19 infection. [146] Camostat isa serine protease inhibitor, historically used in the treatment of pancreatitis, which blocks the entry of SARS-CoV-2 into human cells by interfering with the cellular serine protease TMPRSS2 which is required ACE2 mediated viral entry. [147] Bromhexine is another protease inhibitor undergoing clinical trial in combination with HCQ based on its potential ability to block viral entry. [148] A study in Hong Kong will evaluate the combination of HCQ and interferon β-1b in patients hospitalized with COVID-19 infection [149], based on evidence for efficacy of type I interferons in the treatment of SARS-CoV and MERS-CoV. [150,151] Thus, various medication combinations which include HCQ are currently being investigated for potential efficacy in the treatment of COVID-19.

Considerations for use in the treatment of covid-19

No proven effective therapies for this virus currently exist, however the rapidly expanding knowledge regarding SARS-CoV-2 virology provides a significant number of potential drug targets. Currently, there is no evidence from Randomized Clinical Trials (RCTs) that any potential therapy improves outcomes in patients with either suspected or confirmed COVID-19. There are no clinical trial data supporting any prophylactic therapy. More than 300 active clinical treatment trials are underway.

To perform a literature review, Pubmed/MEDLINE and EMBASE electronic databases were searched using a mix of keywords such as coronavirus, severe acute respiratory syndrome coronavirus 2, 2019-nCoV, SARS-CoV-2, COVID-19 and chloroquine and hydroxychloroquine to identify relevant articles published in English through June 17, 2020. We also searched the largest clinical medicine preprint repository, medRxiv.org. In addition, active clinical trials were identified on ClinicalTrials.gov using the terms hydroxychloroquine and chloroquine in combination with SARS-CoV-2, and COVID-19. The searches as described above resulted in 673 total articles including case reports, review articles, pre-clinical studies, Cochrane reviews, clinical studies, systematic reviews and Meta-analysis reports as well as 18 Controlled and randomized clinical trials, focusing on CQ and HCQ (with or without azithromycin). The authors independently reviewed the titles and abstracts for inclusion. Additional relevant articles were identified from the review of citations referenced.

Evidence for use: review of clinical and preclinical data

Data to support the use of HCQ and CQ for COVID-19 are limited and inconclusive. The drugs have some in vitro activity against several viruses, including coronaviruses and influenza, but previous randomized trials in patients with influenza have been negative. [152,153] In COVID-19, some studies and reports [12,154] demonstrated benefit but had serious methodological flaws, and a follow-up study still lacked a control group. [155] Yet, another very small, randomized study from China in patients with mild to moderate COVID-19 found no difference in recovery rates. [156] Antimalarial drugs can cause ventricular arrhythmias, QT prolongation, and other cardiac toxicity, which may pose particular risk to critically ill persons. [157-177] Given these serious potential adverse effects, the hasty and inappropriate interpretation of the literature.

Data from Solidarity [including the French Discovery trial data], the results from the UK's Recovery trial and review of other evidence on HCQ showed that it does not result in the reduction of mortality of hospitalized COVID-19 patients, when compared with standard of care. These data do not provide solid evidence of increased mortality, though some associated safety signals were reported in the clinical laboratory findings of the add-on Discovery trial, a part of the Solidarity trial. [178, 183] These findings do not apply to the use or evaluation of hydroxychloroquine in pre or post-exposure prophylaxis in patients exposed to COVID-19. When used as post- exposure prophylaxis within 4 days after moderate or high-risk exposure, a prospective randomized trial found that hydroxychloroquine failed to prevent illness compatible with Covid-19 or confirmed infection. [183]

Experimental studies have suggested that CQ has the capability of inhibiting the replication of several intracellular micro-organisms including coronaviruses in-vitro by increasing endosomal pH, interfering with the glycosylation of cellular receptor of SARS-CoV, blocking viral infection [27] and by inhibiting sialic acid biosynthesis, which is used

Table 4: Review of Clinical Data for chloroquine and Hydroxychloroquine in COVID-19.

Source/Study	Study Design	Methods [Study population, inclusion criteria, study period]	Study Description: Drug/ combination or Intervention used in the study	Results/Outcomes of study [Positive and Negative] and conclusion	Adverse Drug Reaction	Reference
Chloroquine and hydroxychloroqu ine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries	Systematic search and narrative review	- Systematically searched the PubMed database using key words CQ AND COVID- 19, and HCQ AND COVID-19	The antiviral activity of CQ and HCQ have been identified in the in- vitro studies	Low cost of CQ and HCQ could be an effective strategy to counter COVID-19 in middle- and low- income countries - Case reports of CQ- induced cardiomyopathy and reversible heart failure pointed to a reduced cardiovascular risk with both these compounds	- Precautions while using both these drugs -include frequent monitoring of hematological parameters, measurement of serum electrolytes, blood glucose and hepatic as well as renal functions	[158]
Aminoquinolines against coronavirus disease 2019 [COVID-19]: chloroquine or hydroxychloroquine	Systematic Review	- Review of articles	CQ and HCQ	HCQ downregulates the expression of Toll- like receptors E6 and TLR-mediated signal transduction HCQ preferred over CQ to its lower ocular toxicity - HCQ decreases the production of interleukin-6 - CQ not as widely available as HCQ in some countries	CQ greater adverse effects than HCQ F5 - CQ can interact with lopinavir/Ritonavir, resulting in prolongation of the QT interval - Retinopathy- dose- limiting effect of HCQ	[159]
Chloroquine and hydroxychloroqu ine in COVID-19 [Use of these drugs is premature and potentially harmful]	Review	An open, Non-randomized study of HCQ	CQ and HCQ	Vaccine or treatment with drugs needed targeting specific structures in the virus Drugs initially supported by evidence may later prove to be more harmful than beneficial - Need properly powered randomized controlled trials of CQ or HCQ - Except for supportive measures, infection with SARS- CoV-2 is “essentially untreatable”	In 15 trials mentioned- endpoints specified in published protocol differed from those reported, results in low dose group not described, and trial stopped prematurely Study of HCQ, reportedly supported efficacy in 20 patients, but trial design was poor and results unreliable: six patients dropped out, measure of efficacy was not a clinical endpoint and assessments were made on day 6 after starting treatment	[160]
Should chloroquine and hydroxychloroqu ine be used to treat CovidD-19? A rapid review	Rapid Review	- Electronic searches in PubMed and Google Scholar were conducted for relevant literature	CQ or HCQ	Limited evidence of in vitro activity On basis of preliminary results, from ongoing clinical trials some countries have incorporated CQ/HCQ into their treatment protocols for certain patients with COVID-19. Presently no follow- up data to support this approach	- Available in vivo empirical data is limited to two studies, small sample sizes, methodological flaws, and conflicting results	[161]
COVID-19: a recommendation to examine the effect of hydroxychloroqu ine in preventing infection and progression	Proposition	Propose HCQ, which exhibits an antiviral effect highly similar to CQ, could serve as a better therapeutic approach - Urgent need to identify effective and safe medical agents to treat COVID-19	HCQ	HCQ inhibits cytokine storm by suppressing T cell activation - Cheaper and more readily available in China - CQ declared medical agent for COVID-19, released by the National Health and Care Commission of China - HCQ and CQ well distributed through whole body - HCQ may confer	Gastrointestinal responses, most common adverse effects - Patients with long- term exposure to CQ suffer from severe side effects, such as retinopathy, circular defects [or bull’s eye maculopathy], diametric defects in the retina and cardiomyopathy	[162]

The QT Interval in Patients with SARS-CoV-2 Infection Treated with Hydroxychloroquine/Azithromycin	Retrospective study Primary purpose: Evaluate the effect of HCQ and Azithromycin on the QT interval and the risk for malignant arrhythmia induction	Aged 63 ± 15 -Confirmed COVID-19 diagnosis -Baseline QTc [ms] 435 ± 24	HCQ and Azithromycin	QTc prolonged significantly in patients treated with HCQ and Azithromycin - In 11% of patients, QTc prolonged to >500 ms, a known marker for high risk of malignant - Baseline QTc not a reliable predictor of severe QTc prolongation in these patients arrhythmia and sudden cardiac death QTc should be followed repeatedly in patients treated with HCQ and Azithromycin, particularly in patients with renal failure, common complication in patients with SARS-CoV-2	4 patients died from multi-organ failure, without evidence of arrhythmia - HCQ and Azithromycin can increase the risk for QT interval prolongation, drug-induced torsades de pointes [TdP], and drug induced-sudden cardiac death - Azithromycin can cause abnormal changes in the electrical activity of the heart - may lead to fatal irregular heart - Patients at risk for developing this condition after treatment with Azithromycin include those with known risk factors such as existing QT interval prolongation etc. rhythm - CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects	[108, 157, 163]
Clinical Outcomes of Hydroxychloroquine in Hospitalized Patients with COVID-19: A Quasi Randomized Comparative Study	Quasi-Randomized Study	#NAME?	HCQ	High dose HCQ in vitro can exhibit mild cyclo-oxygenase inhibition effects - Recommend more judicious prescription of HCQ in setting of SARS-CoV-2 before larger analysis can be completed	Lack of true randomization in study - Significant confounders existed - HCQ at best did not appear to have a beneficial effect - HCQ administration associated with increased need for escalation of respiratory support - No benefits of HCQ on mortality, lymphopenia, or neutrophil-to-lymphocyte ratio improvement	[164]
Towards Optimization of Hydroxychloroquine Dosing in Intensive Care Unit COVID-19 Patients	Cohort Study	Aged ≥ 38 years - Laboratory confirmed SARS-CoV-2 infection treated by HCQ in ICU	HCQ	Steady-state concentrations achieved within weeks and vary from individual to individual - HCQ dosing regimen should be optimized on basis of PK data available in special populations - Propose loading dose of 800 mg once daily on day 1, followed by 200 mg twice daily for 7 days	Dreaded adverse effect for COVID-19 patients is cardiac toxicity - Concentration of 2 mg/L should not be exceeded to avoid ocular toxicity	[165]
Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study	Non-comparative Observational study Primary purpose: Study effectiveness of HCQ and Azithromycin treatment to cure COVID-19 patients and to decrease virus carriage duration	- Confirmed mild COVID-19	HCQ and Azithromycin	All patients improved clinically - Rapid fall of nasopharyngeal viral load was noted - Virus cultures from patient respiratory samples were negative in 97.5% of patients at Day 5 - Patients were able to be rapidly discharged - Beneficial effect of co-administration of HCQ with Azithromycin in the treatment of COVID- 19 contagiousness	Death of one 86 year- old patient Potential to treat and cure patients at an early stage before irreversible severe respiratory complications take hold and to decrease duration of carriage and avoid the spread of the disease	[137]
A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-1	Randomized controlled trial Primary purpose: Evaluate the efficacy and safety of HCQ in the treatment of patients with moderate COVID-19	- Patients with confirmed COVID-19	HCQ and conventional treatments	All patients showed improvement in follow-up examinations - Prognosis of disease with HCQ treatment in moderate patients is good	None reported - Four cases of the HCQ group and 3 cases of the control group had transient diarrhea and abnormal liver function [P>0.05]- CQ or HCQ, sometimes used in effectscombination with anti-diabetic drug metformin, may have serious toxic side-	[108,156]
Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial 2020	Randomized controlled trial Primary purpose: Evaluate the efficacy and safety of HCQ in the treatment of patients with moderate COVID-19	- Patients with confirmed COVID-19	HCQ	Larger proportion of patients with improved pneumonia in the HCQ treatment group compared with control group - Among patients with COVID-19, the use of HCQ could H5significantly shorten TTCR and promote the absorption of pneumonia	None reported pneumonia sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects	[108,166]

No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection	Non- randomized study Primary purpose: Study virologic and clinical outcomes of HCQ and Azithromyci n treatment in patients hospitalized with severe COVID-19	Aged >12 years - Patients with confirmed COVID- 19	HCQ and Azithrom ycin	No evidence of a strong antiviral activity or clinical benefit of the combination of HCQ and Azithromycin for the treatment of our hospitalized patients with severe COVID- 19 - Within 5 days, 1 patient died, 2 were transferred to ICU, 1 patient had therapy discontinued after 4 days due to prolonged QTc interval - On day 5-6, 80% patients still had a positive test for COVID-19 based on nasopharyngeal swab qPCR	Azithromycin can cause abnormal changes in the electrical activity of the heart - may lead to fatal irregular heart rhythm - Patients at particular risk for developing this condition after treatment with Azithromycin include those with known risk factors such as existing QT interval prolongation etc. - CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side- effects		[108,138, 157]
Observational Study of Hydroxychloroquine in Hospitalized Patients with COVID-19	Observation al Study	- Consecutive patients hospitalized with COVID-19	HCQ	HCQ administration was not associated with either a greatly lowered or increased risk of composite end point of intubation or death - results were similar in multiple sensitivity analyses	CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side- effects		[13, 108]
Treatment of Non-severe Confirmed Cases of COVID-19 and Chemoprophyla xis of Their Contacts as Prevention Strategy: a Clus ter Randomized Clinical Trial [PEP CoV-2 Study]	Cluster-Randomized Clinical Trial Primary purpose: Prevention - Prophylaxis	patients who meet the requirements of the New Coronavirus infection Diagnosis – Aged ≥ 18 years - Negative pregnancy test	HCQ	HCQ showed excellent in vitro results - Strong antiviral effects on SARS- CoV-2 infection of primate cells at low concentration - Ongoing study	Toxicity of already formed NAPQI can be enhanced by CQ and HCQ inhibiting lysosomal digestion of injured organelles. This results in cell death and subsequent liver - CQ or HCQ, necrosis sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects		NCT043040 53 [108,168]
Post-exposure Prophylaxis or Preemptive Therapy for SARS-Coronavirus-2: A Pragmatic Randomized Clinical Trial	Randomized Clinical Trial Primary purpose: Treatment	Aged ≥ 18 years - Confirmed COVID-19	HCQ	If post-exposure prophylaxis with HCQ can prevent symptomatic COVID-19 disease - Test if early HCQ therapy can prevent COVID-19 disease progression - Ongoing study	Toxicity of already formed NAPQI can be enhanced by CQ and HCQ inhibiting lysosomal digestion of injured organelles. This results in cell death and subsequent liver necrosis - HCQ normally has a small risk for hepatotoxic effects - CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects		NCT043086 68 [108,168]
Chloroquine/ Hydroxychloroquine Prevention of Coronavirus Disease [COVID-19] in the Healthcare Setting; a Randomized, Placebo- controlled Prophylaxis Study [COPCOV]	Randomized Clinical Trial Primary purpose: Prevention	Aged ≥ 16 years - Not previously diagnosed with COVID-19 - Participant works in healthcare facility OR likely exposed to COVID- 19 infection	CQ or HCQ	Symptomatic COVID-19 infections-compared between CQ or HCQ and placebo groups - Symptoms severity of COVID-19 - compared between two groups using a respiratory severity score - Ongoing study	HCQ normally has a small risk for hepatotoxic effects - CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects		NCT 043035 07 [108,169]
Randomized Controlled Clinical Trials of Lopinavir/Ritona vir or Hydroxychloroquine in Patients With Mil d Coronavirus Disease [COVID-19]	Randomized Clinical Trial Primary purpose: Treatment	Aged ≥ 16 years - Confirmed mild COVID-19	Lopinavir / Ritonavir Or HCQ sulfate	In-vitro studies revealed that lopinavir/Ritonavir and HCQ have antiviral activity against Severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] - Ongoing study	Most patients tolerated short course of Lopinavir/ Ritonavir, although some experienced gastrointestinal upset, deranged liver function, etc.		NC T043076 93 [163]
Trial of Treatments for COVID-19 in Hospitalized Adults [DISCOVERY and SOLIDARITY]	Randomized Clinical Trial Primary purpose: Comparison of safety and efficacy of treatments for COVID-19 in hospitalized adults	Age ≥ 18 year-old - hospitalized for COVID-19	Remdesivir Or Lopinavir/ Ritonavir Or Interferon Beta-1A Or HCQ	HCQ does not result in the reduction of mortality of hospitalized COVID-19 patients, when compared with standard of care	CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side- effects		NC T043159 48 [178,179]

Evaluating the Efficacy of Hydroxychloroq uine and Azithromycin to Prevent Hospitalization or Death in Persons With COVID-19	Randomized Controlled Trial Primary purpose: Evaluate the efficacy of HCQ and Azithromyci n to prevent hospitalizati on or death in persons with COVID-19	Aged ≥ 18 years - Confirmed COVID-19	HCQ and Azithromycin	Stratification will be by "high" versus "low" risk of progression to severe COVID-19, where "high risk" is defined as a person age ≥ 60 years or having at least one of several specified comorbidities - Ongoing Study	Patients at particular risk for developing this condition include those with known risk factors such as existing QT interval prolongation etc. - CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects	NC T043580 68 [157]
Evaluating the Efficacy and Safety of Bromhexine Hydrochloride Tablets Combined With Standard Treatment/ Standard Treatment in Patients With Suspected and Mild Novel Coronavirus Pneumonia [COVID-19]	Randomized Sequential Clinical Trial Primary purpose: Treatment	Aged ≥ 18 years - Confirmed COVID-19	Brom hexine Hydro chlori de	- Ongoing Study	CQ or HCQ, sometimes used in combination with anti-diabetic drug metformin, may have serious toxic side-effects	NCT 042737 63 [108]
Hydroxychloroqu ine in the treatment of outpatients with mildly symptomatic COVID-19: a multi-center observational study	Retrospective observationa l study					

Table 5: Clinical Trials on HCQ and CQ with Synergistic Combinations.

Title	Study Results	Conditions	Interventions	Locations	URL
Chloroquine/ Hydroxychloroquine Prevention of Coronavirus Disease [COVID-19] in the Healthcare Setting	No Results Available	COVID 19 | Coronavi- rus| Acu te Respiratory Illnesses	Drug: Chloroquine or Hy- droxychloroquine| Drug: Placebo Drug: Hydroxy- chloroquine Sulfate Regu- lar doseChloroquine|Drug: Placebo		NCT 043035 07
Post-Exposure Prophylaxis for Asymptomatic SARS-CoV-2 COVID-19 Patients With choloroquine Compounds	No Results Available	SARS-CoV- 2|Coronavirus Infection|Asy mptomatic Condition|CO VID-19	|Drug: Hydroxychloro- quine Sulfate Loading Dose|Drug:	Expo Covid Center, Lahore, Pun- jab, Pakistan|Mayo Hospital, La- hore, Punjab, Pakistan|Pakistan Kidney and Liver Institute, La- hore, Punjab, Pakistan	NCT 043466 67
Hydroxychloroquine,Hydroxychlor oquine,Azithromycin in the Treat- ment of SARS CoV-2 Infection	No Results Available	Coronavirus Infection	Drug: Hydroxychlo- roquine Sulfate|Drug: Azithromycin|Drug: Chlo- roquine Sulfate	Washington University School of Medicine Infectious Dis- ease Clinical Research Unit, Saint Louis, Missouri, United States|Washington University School of Medicine, Saint Louis, Missouri, United States	NCT 043417 27
Efficacy and Tolerability of Hydroxychloroquine in Adult Patients With COVID-19	No Results Available	Coronavirus Infection	Drug: Hydroxychloroquine Sulfate 200 MG [Plaquenil]	Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan City, Taiwan	NCT 043843 80
Prophylaxis of Exposed COVID-19 Individuals With Mild Symptoms Using choloroquine Compounds	No Results Available	Sars- CoV2|Sympto matic Condition|COVID-19	Drug: Hydroxychloroquine Sulfate Regular dose|Drug: Hydroxychloroquine Sulfate Loading Dose|Drug: Chloroquine|Drug: Placebo	Expo Covid Isolation Center / Mayo Hospital, Lahore, Punjab, Pakistan|Mayo Hospital / King Edward Medical University, Lahore, Punjab, Pakistan|Pakistan Kidney and Liver Institute, Lahore, Punjab, Pakistan|Services Hospital, Lahore, Punjab, Pakistan, Hospital Field	NCT 043511 91
Anti-Coronavirus Therapies to Prevent Progression of Coronavirus Disease 2019 [COVID-19] Trial	No Results Available	Coronavirus|Severe Acute Respiratory Syndrome	Drug: Azithromycin|Drug: Hydoxychloroquine or Chloroquine|Drug: Interferon-Beta	Hamilton Health Sciences, Hamilton, Ontario, Canada	NCT 043244 63
Norwegian Coronavirus Disease 2019 Study	No Results Available	Corona Virus Infection	Drug: Hydroxychloroquine Sulfate	Akershus University Hospital, LÃ¸renskog, Norway	NCT 043163 77

Hydroxychloroquine Versus Placebo in COVID-19 Patients at Risk for Severe Disease	No Results Available	Coronavirus	Drug: Hydroxychloroquine| Drug: Placebo	CH Agen, Agen, France|CHU Amiens, Amiens, France|CHU Angers, Angers, France|APHP and 34 more	NCT 04325893
Chloroquine Phosphate Against Infection by the Novel Coronavirus SARS-CoV-2 [COVID-19]: The HOPE Open-Label, Non Randomized Clinical Trial	No Results Available	Pneumonia, Viral|COVID- 19	Drug: UNIKINON [Chloroquine phosphate] 200mg tablets	Divine Providence Hospital "Pammakaristos", Athens, Greece|Athens General Hospital "Hippokrateio", Athens, Greece|Athens General Hospital of Thoracic Diseases "SOTIRIA", 1st University Pulmonary Clinic, Athens, Greece and 2 more	NCT 04344951
PATCH 2&3:Prevention & Treatment of COVID-19 [Severe Acute Respiratory Syndrome Coronavirus 2] With Hydroxychloroquine	No Results Available	Coronavirus|Corona Virus Infection	Drug: Group A HCQ|Drug: Group B Control	ProHealth New York, New York, New York, United States	NCT 04353037
Safety and Efficacy of Hydroxychloroquine Associated With Azithromycin in SARS-CoV2 Virus [Coalition COVID-19 Brasil II]	No Results Available	Coronavirus Infections|Pneumonia, Viral		Instituto de Cardiologia do Distrito Federal, BrasÃlia, Distrito Federal, Brazil|FundaÃ§Ã£o Social Rural de Colatina, Colatina, Espirito Santo, Brazil|Hospital Vera Cruz AS, Belo Horizonte, Minas Gerais, Brazil and 23 more	NCT 04321278
Hydroxychloroquine for COVID- 1	No Results Available	COVID-19, Hydroxychloroquine Sulfate	Drug: Hydroxychloroquine + azithromycin|Drug: Hydroxychloroquine Drug: Hydroxychloroquine Sulfate|Drug: Placebo	Institute for Tropical Medicine, TÃ¼bingen, Germany	NCT 04342221
Austrian CoronaVirus Adaptive Clinical Trial [COVID-19]	No Results Available	COVID-19	Drug: Chloroquine or Hydroxychloroquine| Drug: Lopinavir/ Ritonavir| Other: Best standard of care|Drug: Rivaroxaban|Drug: Thromboprophylaxis| Drug: Candesartan|Drug: non-RAS blocking antihypertensives|Drug: Clazakizumab|Drug: placebo for clazakizumab	Medical University of Innsbruck, Innsbruck, Tirol, Austria|Medical University of Vienna, Vienna, Austria| and 4 more	NCT 04351724
A Pilot Study to Assess Hydroxychloroquine in Patients With SARS-CoV-2 [COVID-19]	No Results Available	COVID- 19|SARS-CoV-2	Drug: Hydroxychloroquine| Drug: Placebo	Oregon Health and Science University, Portland, Oregon, United States	NCT 04363366

Table 6: Pre-clinical Studies on Hydroxychloroquine and Chloroquine with Synergistic Combinations.

Source/Study	Study Design	Methods [Study population, inclusion criteria, study period]	Study Description: Drug/ combination or Intervention used in the study	Results/Outcomes of study [Positive and Negative] and conclusion	Adverse Drug Reaction	Reference
Chloroquine and hydroxychloro quine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries	Systematic search and narrative review	Systematically searched the PubMed database using key words CQ AND COVID-19, and HCQ AND COVID-19 for both in vitro and clinical studies antiviral activity of CQ and HCQ	CQ and HCQ	Low cost of CQ and HCQ could be an effective strategy to counter COVID-19 in middle- and low- income countries - Case reports of CQ- induced cardiomyopathy and reversible heart failure pointed to a reduced cardiovascular risk with both these compounds	- Precautions while using both these drugs -include frequent monitoring of hematological parameters, measurement of serum electrolytes, blood glucose and hepatic as well as renal functions.	[158]

Aminoquinoli nes against coronavirus disease 2019 [COVID-19]: chloroquine or hydroxychloroquine	Systematic Review	Review of articles both in vitro and clinical studies	CQ and HCQ	HCQ downregulates the expression of Toll- like receptors [TLRs] and TLR-mediated signal transduction -HCQ preferred over CQ to its lower ocular toxicity -HCQ decreases the production of interleukin-6 -CQ not as widely available as HCQ in some countries	CQ greater adverse effects than HCQ -CQ can interact with lopinavir/ritonavir, resulting in prolongation of QT interval. the - Retinopathy- dose-limiting effect of HCQ	[159]
Efficacy of chloroquine and hydroxychloro quine in the treatment of COVID-19	Systematic Review	Review of articles both in vitro and clinical studies	CQ and HCQ	Chloroquine AND hydroxychloroquine have antiviral characteristics in vitro. - findings support the hypothesis that these drugs have efficacy in the treatment of COVID-19	None reported	[175]
Chloroquine for the 2019 novel coronavirus SARS-CoV-2	Narrative Review	Review of in-vitro studies on anti-viral effect of chloroquine	CQ	chloroquine has been shown to be effective in vitro against a broad range of viruses - possible use both in prophylaxis in people exposed to the novel coronavirus and as a curative treatment should be evaluated	None reported	[170]
Chloroquine and hydroxychloro quine as available weapons to fight COVID-19	Narrative Review	Review of in-vitro and clinical studies on anti-viral effect of both chloroquine and HCQ	CQ and HCQ	chloroquine has been shown to be effective in vitro against a broad range of viruses - chloroquine showed - absence of severe side effects - effectivenes s CQ and HCQ, against coronavirus es probably due to alkalinisation of phagolysosome	None reported	
A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19.	Systematic review	Review of in-vitro and clinical studies on effect of CQ and HCQ on SARS-CoV-2	CQ	Chloroquine is a widely used, safe and H4cheap, effective in viral infections in pre-clinical studies. - Specific pre-clinical evidence and expert opinions suggest potential use against SARS-CoV-2.F4	- There is a urgent need of high- quality clinical data from different geographic areas.	[177]
In-vitro study Primary purpose: [1] investigate the antiviral and prophylactic activity of hydroxychloro quine and chloroquine in vitro, with different dosing regimens using the developed PBPK models.	pharmacological activity of chloroquine and hydroxychloroqu ine was tested using SARS- CoV-2–infected Vero cells. - Physiologically based pharmacokinetic [PBPK] models were implemented for both drugs separately	HCQ		Hydroxychl oroquine [EC50 = 0.72μM] was found to be more potent than chloroquine [EC50 = 5.47]- immunomodulatory effect of hydroxychlo roquine also may be	HCQ has better tolerance profile than CQ has better tolerance profile than CQ	[11]
				useful in controlling the cytokine storm that occurs late phase in critically ill patients with SARS-CoV- 2 μM] in vitro for inhibition of SARS-CoV- 2.		
In-vitro study Primary purpose: evaluate the antiviral efficiency of ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine, remdesivir and favipiravir against a clinical isolate of SARS-CoV-2	Vero cells were infected with SARS-CoV-2 at an MOI of 0.05 and treated with different doses of the 7 indicated antiviral drugs for 48h. The viral yield in the cell - Standard assays were carried out to measure the effects of these compounds on the cytotoxicity, virus yield and infection rates		Drug: CQ Drug: RemedesvirDrug: Ribavirin Drug: PenciclovirDrug: NitazoxanideDrug: Nafamostat Drug: Favipiravir	remdesivir and chloroquine are highly effective in the control of 2019-	CQ or HCQ, sometimes used in combination with anti- diabetic drug metformin, may have serious toxic side-effects	[27,108]

			nCoV infection in vitro				
Hydroxychlor oquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2	In vitro study Primary purpose: [1] Comparison of antiviral effect		cytotoxicit y of HCQ AND CQ in African green monkey kidney VeroE6 cells [ATCC-	HCQ and CQ	50% cytotoxic concentratio n [CC50] values of CQ and HCQ were 273.20 and 249.50 μM,	- results show that HCQ can efficiently inhibit SARS-CoV-	[60]
			1586] was measured by standard CCK8		respectively, which are not significantly different from each other. - at all MOIs, the 50% maximal effective concentration [EC50] for CQ was significantly lower than that of HCQ. - selectivity index [SI = CC50/E C50] of CQ		
			assay - the dose– response curves of		was higher than that of HCQ - results were corroborated by immunofluo rescence microscopyof expression levels of virus nucleoprotei n after		
			the two compounds against SARS- CoV-2		treatment. - both CQ and HCQ		
			were determined at four different multipliciti es of		blocked the transport of SARS-CoV-		
			infection [MOIs] by quantificati on of viral RNA copy numbers in the cell supernatant at 48 h post infection. - expression levels of virus nucleoprot ein [NP] at the indicated drug concentrati ons at 48 h p.i determined by		2 from EEs to ELs. which appears to		
			immunofluorescence quantificati on analysis. co-localization of virions with early endosomes [EEs] or endolyso-somes [ELs] was		be a		
			analyzed by immunofluoresce nce analysis		requirement to release		
			[IFA] and confocal microscopy.		the viral genome as in the case of SARS-		
					CoV. CQ		
					treatment did not		
					cause obvious changes in the number and size of ELs;		
					however, the regular vesicle structure seemed to be disrupted, at least partially. By contrast, in HCQ-		
					treated cells, the size and number of ELs increased significantly.		
Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection	In-silico study	combination of structural and molecular modelling approaches to study ef-fect D6of CQ and HCQ on ability of SARS- CoV-2 S proteins to host cell surface gangliosides	HCQ and CQ	new mechanism of action of chlo-roquine and hydroxychlo roquine against SARS-CoV-2 infection - CQ and HCQ bind sialic acids and gan-gliosides with high affinity - in the presence of CQ and HCQ the viral S protein is no longer able to bind gangliosides. - The study data sup-port the use of CQ, and preferentiall y HCQ as initial therapy for patients infected with SARS- CoV-2.	The study data support the use of CQ, and preferentiall y HCQ as initial therapy for patients infected with SARS-F8 CoV-2.	[9]
Finding the do se for hydroxy chloroquine pr ophylaxis for COVID-19; the desperate s earch for effec tiveness.	In-silico Simulation	Simulation model code including population phar-macok inetics parameters - Simulation model code including population phar-macok inetics parameters - Simulation R code for pre- exposure prophylaxis	HCQ	Simulations suggest higher treat-ment and prophylactic doses for COVID-19. - Found higher dosing likely be required in pre- exposure setting, while post-exposure - clini-cal trials areconcentrations were not maintained as longregimen target needed to establish safety and efficacy.	Five days of 400mg without loading dose was not sufficient to maintain daily troughs above the EC50 in 50% of the subjects for 14 days. - Patients with active COVID-19 disease may require a different efficacy target due to higher viral loads carefully monitored. - HCQ systemic severe adverse effects, toxicities are mostly associated with daily, long-term use.	[11]

Optimizing Hydroxychloroquine Dosing for Patients With COVID-19: An Integrative Modeling Approach for Effective Drug Repurposing.	In-silico Simulation	Integrate historic and emerging pharmacol ogical and toxicity data to understand safe and efficacious HCQ dosing strategies for COVID-19 treatment. - data sources included were [i] longitudina l clinical, pharmacok inetic [PK], and virologic data from patients with severe acute respiratory syndrome-2 [SARS- CoV-2] infection who received HCQ with or without azithromyc in - [ii] in vitro viral replication data and SARS-CoV-2 viral load inhibition by HCQ, [iii] a population PK model of HCQ, and [iv] a model relating chloroquine PKs to corrected QT [QTc] prolongation.	Drug: HCQ Drug: HCQ and Azithromycin	A mechanistic PK/virologic/QTc model for HCQ was developed and externally validated to predict SARS- CoV-2 rate of viral decline and QTc prolongation - data supports high- dose HCQ regimens [> 400 mg b.i.d. for ≥ 5 days] to be used for most effective treatment of patients with COVID-19 - HCQ doses > 600 mg b.i.d. were also predicted to prolong QTc intervals. - Pharmacological rationale and dosing tools for use of HCQ in patients with COVID-19 can be used to rationalize and utilize use of this medicine in the current pandemic. Evaluation of higher HCQ doses is needed to ensure adequate safety and efficacy.	Low doses of HCQ [e.g., 400 mg q.d.] might not offer substantial benefit - HCQ doses > 600 mg b.i.d. were also predicted to prolong QTc intervals - clinical implications warranting further safety assessment.	[11]

as a receptor by human coronaviruses, that makes this agent a broad antiviral agent. Furthermore, CQ inhibits MAP-kinase, interfering with SARS-CoV-2 molecular crosstalk, besides altering the virion assembly, budding and interfering with the progress of the infection. [170] Previous experimental studies have also demonstrated that CQ has potent anti-SARS-CoV-1 effects in vitro, primarily attributable to a deficit in the glycosylation receptors at the virus cell surface, so that it cannot bind to the Angiotensin-Converting Enzyme 2 (ACE2) expressed in lung, heart, kidney and intestine. Since SARS-CoV-2 utilizes the similar surface receptor ACE2, it is believed that CQ can also interfere with ACE2 receptor glycosylation thus prevents SARS-CoV-2 attachment to the target cells. [27,170-172] Other studies on the effect of CQ in vitro (using Vero E6 cell line infected by SARS-CoV-2) foundCQ to be highly effective in reducing viral replication that can be easily achievable with standard dosing due to its favorable penetration in tissues including the lung. [172,173]

Since the structure and mechanism of action of CQ and HCQ are identical except an additional hydroxy moiety in one terminal in HCQ, both act as a weak base that can change the pH of acidic intracellular organelles including endosomes/ lysosomes, it is believed that both the agents could be effective tools against SARS-CoV-1 and SARS- CoV-2. [6,173] Some data show HCQ effectively inhibited both the entry, transport and the post-entry stages of SARS-CoV-2, like CQ, and one study found HCQ to be a more potent agent than CQ in inhibiting SARS-CoV-2 in vitro. [11,60] Addition of hydroxyl molecule makes HCQ less permeable to blood-retinal barrier and allows faster clearance from retinal pigment cell, thereby suggesting a lesser risk of retinal toxicity with HCQ, as compared to CQ. [174] Furthermore, the narrow therapeutic and safety index margin with CQ makes HCQ a safer option than CQ. CQ and HCQ have shown therapeutic activity or immune modulatory effects in a wide range of other viral diseases and are believed to have the same effect on SARS-COV2 Patients. Some studies indicated that CQ and HCQ were used as initial therapy for patients with SARS-COV2 infection. [9] In addition, another study revealed that higher treatment and prophylactic doses for COVID-19 were needed than those recommended for malaria. Therefore, patients with active COVID-19 disease may require a different efficacy target due to higher viral loads, as Low doses of HCQ (e.g., 400 mg q.d.) might not offer substantial benefit. [11] However, Using HCQ and CQ in higher doses to cure patients with SARS-COV2 can only yield severe and life-threatening complications, adverse effects, and neurotoxicity. [11] These adverse effects are gastrointestinal upset, nausea, vomiting, diarrhea, and Retinopathy and the eventual risk of ventricular arrhythmias from prolonged use. [176]

Combination therapy with HCQ and CQ also have toxic effects on patients with underlying health conditions. For example, CQ or HCQ, sometimes used as a combination drug with the anti-diabetic drug metformin, may have severe toxic side effects. [27, 108]

Furthermore, the Development of QTc prolongation is of particular concern related to the treatment of COVID-19 and has been reported more frequently in Covid-19 patients, especially when HCQ is given in combination with azithromycin. [42]

The goal of the Repurposed Antiviral Drugs for COVID-19 - Interim WHO Solidarity Trial Results [1] was to assess the safety and efficacy of four drugs [remdesivir, hydroxychloroquine, lopinavir, interferon] among patients hospitalized with the virus. Patients were randomized in an open-label fashion equally to either remdesivir (n = 2,750), hydroxychloroquine (n = 954), lopinavir without interferon [n = 1,411], interferon with or without lopinavir (n = 2,063), or no trial drug (n = 4,088). The intravenous regimen for remdesivir was 200 mg on day 0 and 100 mg on days 1-9. The oral regimen for hydroxychloroquine was four tablets at hour 0, four tablets at hour 6, and, starting at hour 12, two tablets twice daily for 10 days.

Each tablet contained 200 mg of hydroxychloroquine sulfate. The regimen for lopinavir was two tablets twice daily for 14 days. Each oral tablet contained 200 mg of lopinavir [plus 50 mg of booster ritonavir]. The regimen for interferon (mainly subcutaneous) was three doses over a period of 6 days (the day of randomization and days 3 and 6) of 44 μg of subcutaneous interferon beta-1; where intravenous interferon was available, patients receiving high-flow oxygen, ventilation, or Extracorporeal Membrane Oxygenation (ECMO) were instead to be given 10 μg intravenously daily for 6 days.

The study design consisted of a total of 11,266 (81%: <70 years; female: 38%) participants enrolled with a 28-day duration of follow-up. The primary outcome was in-hospital mortality. The interim principal findings (below) showed the remdesivir,

hydroxychloroquine, lopinavir, and interferon regimens had little or no effect on hospitalized patients with COVID-19, as indicated by overall mortality, initiation of ventilation, and duration of hospital stay. None of the subgroup analyses tested were significant.

Based on the results of the SOLIDARITY trial, a recent mortality study of four repurposed antiviral drugs conductedby WHO, on a total of 11,330 patients signed up from 405 hospitals in 30 countries to evaluate the effect of HCQ) being one of the four) on hospitalized patients with Covid-19, as indicated by overall Mortality, initiation of ventilation, and duration of hospital stay, HCQ was considered to be neither effective, nor safe to be used as an anti- viral drug at the dosage tested. The rate ratio for Death over a 28-day period for HCQ compared with standard care is 1.19 (95% CI, 0.89 to 1.59), suggesting that HCQ had little or no effect on in-Hospital Mortality. A stratified rate ratio for in-Hospital Death subdivided by age and respiratory support at trial entry in patients given HCQ vs. Controlgroup suggested that HCQ had no definite effect on Mortality (P> 0.10). Statistical results of 104 of 947 patients receiving hydroxychloroquine and 84 of 906 receiving its control was rate ratio, 1.19; 95% CI, 0.89 to 1.59; P=0.23. Ventilation was later initiated after the randomization of 295 patients, with 75 patients receiving HCQ and 66 receiving its control. [179] This data reinforces null findings in the Randomized Evaluation of Covid-19 Therapy (RECOVERY) trial using HCQ. [180] For HCQ, the joint rate ratio for Death (combining the Solidarity and RECOVERY trials) was 1.10 (95% CI, 0.98 to 1.23), with no apparent benefit whether the patient was receiving ventilation or not, ruling out any material benefit from this hydroxychloroquine regimen in hospitalized patients with Covid-19.

Most recent results from the WHO Solidarity randomized trial and updated meta-analysis [2] indicated an in- hospital mortality for remdesivir (n=4.146) or control (n=4,129); 14.5% vs 15.6% (p=0.12) with a reported non- prespecified composite of death or progression to ventilation: 19.6% vs. 22.5% (RR 0.84, 95% CI 0.75-0.93, p= 0.001).

The significant decrease in the production of pro-inflammatory markers and cytokines [120] with HCQ has made this agent a successful disease modifying anti-inflammatory agent in the treatment of various autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus and Sjogren's syndrome. [30] This anti-inflammatory property of HCQ gives it the potential to reduce the cytokine -induced damage associated with COVID-19. Long- term clinical safety profile of HCQ is better than that of CQ, that allows higher daily dose of HCQ with less drug- drug interactions. [158,175]

Among hospitalized COVID-19 patients, observational studies have noted that hydroxychloroquine exposure has not been associated with a reduction in the risk of death. [13,55, 178-182] When used as post-exposure prophylaxis within 4 days after moderate or high-risk exposure, a prospective randomized trial found that hydroxychloroquine failed to prevent illness compatible with Covid-19 or confirmed infection. [183] However, an observational study from Michigan, reported improved survival when hydroxychloroquine was administered within 2 days of hospitalization [184], while a later retrospective observational study of SARS-CoV-2 infected non- hospitalized mildly symptomatic patients' HCQ exposure was associated with a decreased rate of subsequent hospitalization with no reported arrhythmia events. [185] Therefore, further investigation of HCQ use in the mildly symptomatic outpatient population is needed.

Conclusion

CQ and HCQ have been used for many years in the treatment and prophylaxis of uncomplicated malaria caused by susceptible Plasmodium parasites. They have also been used in many inflammatory conditions involving the immune and related systems. CQ and HCQ are cheap and safe drugs that have been used for many years and are potentially clinically applicable against the SARS-CoV-2. The current global interest in their potential use to save lives during the ongoing COVID-19 pandemic stems from the elucidated structural similarities between the causative agent SARS-CoV-2 and the coronaviruses causing the 2002-2003 SARS outbreak and the Zika virus disease of 2015-2016. CQ and HCQ played important promising roles in the management of these latter coronavirus-causing diseases and hence attention is being given to study and understand the conditions and the patient selection needed for their safe and effective use in COVID-19 treatment. But based on the evidence obtained in recently reported studies, from both in vitro studies and clinical studies, there is no credible evidence on the efficacy and safety of these agents in COVID-19.

This narrative review is an important contribution to the current literature on CQ and HCQ as it comprehensively consolidates the vast amount of information on the pharmacological activity, pharmacokinetic properties, and the clinical uses, limitations, and adverse effects of these two drugs. This article also summarizes current evidence regarding use of HCQ and CQ alone, or in combination with other drugs for treatment, repurposed or experimental, for COVID-19 and provides an overview of current clinical experience and treatment guidance for the repurposed use of these two drugs for treatment of this COVID-19 pandemic.

Overall, results of the data reported in major trials indicates that among patients admitted with COVID-19, neither HCQ, nor CQ or their combinations demonstrated a mortality benefit compared with their respective controls. But a major limitation is that large trials like SOLIDARITY was that it was an open-label study with no placebo control. Longer-term data are expected however, the negative overall findings from the regimens tested are adequate to rebutearly anticipations that any of these regimens will substantially reduce inpatient mortality, the initiation of mechanical ventilation, or hospitalization duration. However, there are studies that show contribution of HCQ treatment towards improved outcomes in early stage of hospitalization and in non-hospitalized Covid-19 patients. Therefore, additional exploration of hydroxychloroquine in this mildly symptomatic population is warranted. Despite the massive amount of data-based evidence reported on COVID-19, the available evidence cannot be considered conclusive, due to the methodological flaws in the studies. The quality of ongoing and planned clinical research has to be urgently upgraded in order to generate more conclusive data about the effectiveness

of HCQ and CQ in treatment of COVID-19, and on their use in pre or post-exposure prophylaxis in patients exposed to COVID-

19. Until then, use of these two drugs, alone or in association with other synergistic therapies, needs to be consideredjudiciously.

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