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An overview of antiviral strategies for coronavirus 2 (SARS-CoV-2) infection with special reference to antimalarial drugs chloroquine and hydroxychloroquine

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Abstract

At present, neither specific antiviral drugs, nor vaccine is recommended for coronavirus disease 2019 (COVID-19) treatment. In this review we discuss the drugs suggested as therapy for COVID-19 infection, with a focus on chloroquine and hydroxychloroquine. The list of drugs used for COVID-19 treatment includes a combination of lopinavir and ritonavir, remdesivir, favipiravir, alpha-interferon, ribavirin, atazanavir, umifenovir, and tocilizumab. As their efficacy and safety are under investigation, none of the regulatory agencies approved them for the treatment of COVID-19 infection. Although chloroquine and hydroxychloroquine possess antiviral and immunomodulatory effects, in practice benefit of their use for COVID-19 treatment is controversial. Several studies investigating hydroxychloroquine were stopped and the French national medicines regulator suspended its use in clinical trials because of safety concerns. The results from the double-blind, randomised clinical trials, including large number of participants, will add better insight into the role of these two drugs as already available and affordable, antimalarial therapy. The ethical issue on emergency use of chloroquine and hydroxychloroquine in the settings of COVID-19 should be carefully managed, with adherence to the "monitored emergency use of unregistered and experimental interventions" (MEURI) framework or be ethically approved as a trial, as stated by the WHO. Potential shortage of chloroquine/hydroxychloroquine on the market can be overbridged with regular prescriptions by medical doctors and national drug agency should ensure sufficient quantities of these drugs for standard indications.

Abbreviations: ARDS, acute respiratory distress syndrome; ACE-2, angiotensin-converting enzyme 2; b.i.d., twice a day; CDC, Centre for Disease control and Prevention; CHM, the United Kingdom Commission on Human Medicines; COVID-19, coronavirus disease 2019; CRS, cytokine release syndrome; CQ, chloroquine; DRESS syndrome, drug rash with eosinophilia and systemic symptoms syndrome; ECG, electrocardiogram; EMA, European Medicines Agency; FDA, Food and Drug Administration; HCQ, hydroxychloroquine; IC_{50}, half-maximal inhibitory concentrations; IL, interleukin; IU, international unit; lapp, referring to Sámi people, indigenous Finno-Ugric people; LDH, lactate dehydrogenase enzyme; n/a, not available; NIH, National Institutes of Health; *p.o.*, *per os*; q.d., once a day; SARS-CoV-2, severe acute respiratory syndrome corona virus 2; SIRS, systemic inflammatory response syndrome; *s.c.*, subcutaneously; SOC, standard of care; t.i.d., three times a day; WHO, World Health Organization.

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1 | INTRODUCTION

A coronavirus is a group of highly diverse, enveloped, positive-sense, and single-stranded RNA viruses that cause respiratory tract infection.[1,2] Coronaviruses are common in animals; some of them can be transmitted into humans, such as a novel coronavirus.[1] The first cases of coronavirus disease 2019 (COVID-19), caused by a severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), were recognised in Wuhan, Hubei Province, China, in December 2019.[3,4] Common signs of infection include respiratory symptoms, fever, and cough.[1,56] The illness is ranging from the common cold to more severe disease, such as pneumonia, acute respiratory distress syndrome (ARDS), and complications caused by systemic hyperinflammation.

Genetic sequencing of the virus suggests that SARS-CoV-2 is a beta coronavirus closely linked to the SARS virus.[4] The COVID-19 infection has higher levels of transmissibility and pandemic risk than that of SARS-CoV.[1] The entry receptor utilised by SARS-CoV-2 and SARS-CoV is Angiotensin-Converting Enzyme 2 (ACE-2) receptor.[2]

Recent research reported that SARS-CoV-2 likely originated in bats, based on the similarity of its genetic sequence to that of other CoVs. The intermediate animal species of SARS-CoV-2 between a bat and humans is still unknown.[7] Human-to-human transmission happens mainly by the respiratory route or through contact with infected secretions.[2] Asymptomatic individuals transmit the virus.[8] Based on data from cases in Wuhan, the incubation time ranges from 3 to 7 days, up to 2 weeks.[8]

Review criteria

- This review is intended to be a narrative experts based review: a focused evaluation of preeminent papers about the drugs suggested as therapy for COVID-19 infection, with a focus on chloroquine and hydroxychloroquine.

Message for the clinic

- **In this review we discuss the drugs suggested as therapy for COVID-19 infection, with a focus on chloroquine and hydroxychloroquine. There is rationale from pre-clinical evidence of these drugs effectiveness and their safety from long-time clinical use in other indications. Data from clinical trials are urgently needed to establish the position of these drugs in COVID-19 infection treatment.**

2 | CORONAVIRUS DISEASE 2019

2.1 | Pathogenesis

COVID-19 is capable of producing an excessive immune reaction in humans resulting in extensive tissue damage, known as a "cytokine release syndrome" (CRS).[8] The SARS-CoV-2 binds to alveolar epithelial cells, activates innate and adaptive immune response, leading to the release of various cytokines, including interleukin (IL)-6.[9] Proinflammatory factors increase vascular permeability, and this leads to the appearance of blood cells and fluid in alveoli, resulting in dyspnea and respiratory failure.

Recent findings indicate that SARS-CoV-2 bounds to heme groups on hemoglobin molecule, separate iron with consequent dissociation of heme into porphyrin, which is "captured" by virus proteins.[10] Attack of oxidised hemoglobin by viral proteins will decrease the quantity of hemoglobin that can carry oxygen and carbon dioxide. This study found that viral non-structural proteins (ORF1ab, ORF10, and ORF3a) can simultaneously attack the heme on the 1-beta chain of hemoglobin to dissociate the iron leading to a formation of the porphyrin.[10] Additionally, E2 glycoprotein, envelope protein, nucleocapsid phosphoprotein, ORF1ab, ORF7a, and ORF8 of the virus could bind to porphyrin.[10] In summary, the virus is in a massive demand for porphyrin to survive.

2.2 | Clinical course

The treatment depends on the clinical stage of the disease.[6,11-15] The report from the Chinese Centre for Disease Control and Prevention analysed 44,672 confirmed cases of COVID-19 and described three clinical stages.[6,16] Stage I covers mild disease that usually refers to early infection and is present in 81% of patients.[6] The clinical symptoms of stage I are fever, fatigue, cough, anorexia, malaise, muscle pain, sore throat, dyspnea, nasal congestion, headache, diarrhoea, nausea, and vomiting. Prominent clinical signs include lymphopenia, increased prothrombin time, elevated values of D-dimer, and lactate dehydrogenase enzyme (LDH).[12]

Stage II is a severe (pulmonary) phase found in 14% of patients.[6] Based on clinical presentation, there are two types of this stage IIa and IIb. Stage IIa is characterised by pneumonia without hypoxia and no need for supplemental oxygen. While in stage IIb patients have severe pneumonia with hypoxia, fever, or suspected respiratory infection. Clinical presentation includes respiratory rate >30 breaths/min; severe respiratory distress; or SpO2 ≤93% on room air, hypoxia PaO2/FiO2a ≤ 300 mmHg or >50% of lung involvement on imaging within 24 to 48 hours[6]; transaminitis, low-normal procalcitonin level.[12]

The critical stage III, known as systemic hyperinflammation, is found in 5% of patients.[6] In this stage, patients are critically ill with ARDS, systemic inflammatory response syndrome (SIRS) or shock, cardiac failure, PaO2/FiO2 <150 mm Hg, elevated inflammatory markers (CRP, LDH, IL-2, IL-6, IL-7, D-dimer, ferritin), troponin and

NT-proBNP elevation.[12] The case-fatality rates vary between countries, ranging from 0.1 % to 27.5%.[15]

3 | PHARMACOTHERAPEUTIC APPROACH

3.1 | Antiviral medications used for COVID-19 treatment

At present, no pharmacological agent, except remdesivir, has been approved by regulatory agencies for the treatment of COVID-19. Medical practitioners need a critical analysis of drugs proposed to be effective against COVID-19. So far, the recommended treatment for patients with severe COVID-19 is symptomatic, as supportive treatment interventions with oxygen therapy and different modes of mechanical ventilation. The World Health Organization (WHO) released a document summarising guidelines and scientific evidence based on the treatment of previous epidemics caused by a human coronavirus.[13,17] The numerous antiviral drugs with different mechanisms of action are explored in clinical trials for COVID-19 treatment (Table 1) (Figure 1). Amongst the listed drugs, chloroquine and hydroxychloroquine are the most examined and used drugs in the current outbreak of SARS-CoV-2 (Table 1). The majority of pharmacotherapeutic guidelines for COVID-19 treatment suggest the use of chloroquine and hydroxychloroquine.[38-41]

3.2 | Chloroquine and hydroxychloroquine

3.2.1 | Antiviral activity of chloroquine and hydroxychloroquine in vitro

An old drug for malaria treatment, chloroquine, has been in clinical use since 1944.[42] Chloroquine is a weak base, which concentrates on the highly acidic digestive vacuoles of susceptible Plasmodium, where it binds to heme and disrupts heme sequestration. Hydroxychloroquine (β-hydroxylated N-ethyl substituents of chloroquine) was synthesised in 1946 and introduced in clinical use as equivalent to chloroquine in chemoprophylaxis or treatment of acute attacks of malaria.[42] Chloroquine and hydroxychloroquine belong to disease-modifying antirheumatic drugs used in dermatology and rheumatology.[43] Additionally, hydroxychloroquine is approved by the Food and Drug Administration (FDA) for treating lupus erythematosus.[43]

Both drugs, chloroquine, and hydroxychloroquine, possess the immunomodulatory effects. They increase pH within intracellular vacuoles, therefore, change the ability of acid hydrolases and molecular assembly needed for antigen peptide processing.[43] Finally, this will lead to decreased stimulation of autoimmune CD4+ T cells and downregulation of autoimmune responses. Moreover, both drugs reduce the secretion of proinflammatory cytokines, particularly TNFα in many cells, including human peripheral blood mononuclear cells and human blood.[44]

Researchers suggest that chloroquine inhibits viral replication, TNFα, and IL 6 production and the subsequent cascade of events leading to ARDS.[45,46] In vitro, chloroquine is an effective inhibitor of the SARS-CoV replication.[47,48] Chloroquine half-maximal inhibitory concentrations (IC_{50}) for SARS-CoV in vitro inhibition in Vero E6 cells are 1000-fold below the human plasma concentrations of chloroquine following acute malaria treatment with this drug in a dose of 25mg/kg, over 3 days.[48] Since the chloroquine dosage used for the treatment of rheumatoid arthritis (3.6 mg/kg) produced the same plasma as the IC_{50} for the inhibition of SARS-CoV, one study claimed that chloroquine should be considered for immediate use in the prevention and treatment of SARS-CoV infections.[48]

Results from the in vitro studies investigating chloroquine antiviral activities showed compromised virus/cell fusion with increased endosomal pH and interference with the terminal glycosylation of cellular ACE-2 receptors.[49] These changes result in strong chloroquine antiviral effects on SARS-CoV infected primate cells. Decreased glycosylation of ACE-2 receptors lowers their affinity for SARS-CoV spike (S) protein, potentially preventing infection.[49] Moreover, reduced virus-endosome fusion generated by the rapid elevation of endosomal pH may be an antiviral mechanism under post-treatment conditions.[47] Based on in vitro studies, chloroquine has prophylactic and therapeutic antiviral activity.

Although SARS-CoV-2 S-protein possesses a weaker binding affinity for ACE-2 receptors than the corresponding protein of SARS-CoV, it still maintains a strong binding affinity to human ACE-2 receptors to result in significant risk for human transmission.[9,50] One of the studies tested the pharmacological activity of chloroquine using SARS-CoV-2 infected Vero E6 cells.[34] The use of time-of-addition assay demonstrated that chloroquine influenced both entry and post-entry stages of the SARS-CoV-2 infection in Vero E6 cells.[51] Chloroquine EC_{90} value was 6.90 μM, which equals plasma levels measured in patients with rheumatoid arthritis treated with 500 mg daily dose.[51] The chemical components in chloroquine phosphate compete with the porphyrin and bind to the viral proteins, preventing the attack of viral proteins on heme or binding to the porphyrin.[10] The chloroquine can inhibit E2 glycoprotein and non-structural protein ORF8 from binding to the porphyrin.[10] Additionally, chloroquine can prevent viral ORF1ab, ORF3a, and ORF10 attack on the heme to form the porphyrin.[10]

In vitro, hydroxychloroquine exhibited lower EC_{50} values than chloroquine, indicating that the former has a more potent antiviral activity.[52] Dosing recommendations for hydroxychloroquine rely on a physiologically based pharmacokinetic model.[52] Oral application of hydroxychloroquine sulfate (a loading dose of 400 mg given twice daily, followed by 200 mg twice daily, for 4 days) reached three times the potency of chloroquine phosphate when given 500 mg twice daily for 5 days (Table 2).[52] Based on the superior antiviral and prophylactic activity of hydroxychloroquine in vitro study, the authors suggested that hydroxychloroquine in combination with anti-inflammatory drugs might be used in the treatment of severe SARS-CoV-2 infection.[52]

TABLE 1 Medications with antiviral activity proposed for the treatment of COVID-19, dosing, clinical stage of the disease planned for their use, safety issues (adverse effects and contraindications), and described interactions relevant to their applications[11,18-37]

Chloroquine phosphate Chloroquine phosphate 500 mg = chloroquine base 300 mg	• Mechanism of action[a] • Dosing[b] • COVID-19 Clinical stage: ○ I, IIa, III • Safety: ○ Not clearly defined adverse effects for the doses studied for COVID-19. Cited: cardiac arrhythmia, cardiomyopathy, ECG changes, hypotension, delirium, depression, extrapyramidal reaction, headache, polyneuropathy, pruritus, urticaria, rash, exfoliative dermatitis, hypoglycemia, abdominal cramps, diarrhoea, nausea, vomiting, hepatitis, increased liver enzymes, agranulocytosis, aplastic anaemia, hemolytic anaemia, anaphylaxis, angioedema, DRESS syndrome, ophthalmic disorder, deafness ○ Contraindications[c]: known hypersensitivity to chloroquine or any of the excipients • Interactions[d]: ○ dextropropoxyphene, amiodarone, flecainide, bepridil, mexiletine, rifampicin, sulfadiazine, carbamazepine, phenobarbital, phenytoin, primidone, ziprasidone, St John's wort
Hydroxychloroquine	• Mechanism of action[a] • Dosing[b] • COVID-19 Clinical stage: ○ I, IIa, III • Safety: ○ Not clearly defined adverse effects for the doses studied for COVID-19. Cited: Retinopathy, bronchospasm, acute hepatic failure, exacerbation of porphyria, cardiomyopathy, severe hypoglycemia, bone marrow suppression ○ Contraindications[c]: Known hypersensitivity to 4-aminoquinoline compounds, hypersensitivity to chloroquine or any of the excipients, pre-existing maculopathy of the eye, children aged <6 years of age, lapp lactase deficiency or glucose-galactose malabsorption; weight <35 kg • Interactions[d]: ○ dextropropoxyphene, metamizole, amiodarone, flecainide, bepridil, mexiletine, rifampicin, sulfadiazine, carbamazepine, phenobarbital, phenytoin, primidone, ziprasidone, St John's wort
Lopinavir; ritonavir	• Mechanism of action: ○ Inhibition of 3-chymotrypsin-like protease • Dosing: ○ 400 mg/100 mg b.i.d. , *p.o.* 10 days • Clinical stage: ○ IIb, III • Safety: ○ Adverse effects: respiratory tract infection, skin infection, anaemia, leucopenia, neutropenia, allergic reactions, headache, dizziness, diabetes mellitus, hypertriglyceridemia, hypercholesterolemia, hypertension, hepatitis, pancreatitis, myalgia ○ Contraindications[c]: Hypersensitivity to the drug, contraindicated in children <14 days, pregnant women, severe hepatic insufficiency, renal failure, and patients treated with disulfiram or metronidazole due to the potential risk of toxicity from the excipient propylene glycol • Interactions[d]: ○ dextropropoxyphene, amiodarone, bepridil, disopyramide, dofetilide, flecainide, quinidine, rifampicin, apixaban, clopidogrel, rivaroxaban, ticagrelor, eplerenone, ivabradine, lercanidipine, ranolazine, sildenafil, pimozide, quetiapine, ziprasidone, midazolam (oral), triazolam, cisapride, domperidone, sirolimus, lovastatin, simvastatin, budesonide, fluticasone, mometasone, triamcinolone, St John's wort
Remdesivir	• Mechanism of action: ○ Formally known as GS-5734, is monophosphate prodrug, leading to active C-adenosine nucleoside triphosphate analogue, which incorporates into nascent viral RNA chains and results in premature termination. • Dosing: ○ 200 mg i.v. the first day, then 100 mg *i.v.* next 9 days (total duration 10 days) • Clinical stage: ○ III • Safety: ○ Remdesivir is an investigational medicinal product only available on a use basis direct from the manufacture • Interactions[d]: ○ rifampicin, rifapentine, carbamazepine, phenobarbital, phenytoin, primidone, St John's wort, adrenaline (epinephrine), dobutamine, noradrenaline, vasopressin

(Continues)

TABLE 1 (Continued)

Favipiravir	• Mechanism of action: ○ Also known as T-705 is a prodrug of a purine nucleotide, favipiravir ribofuranosyl-5'-triphosphate. It inhibits the RNA polymerase and halts viral replication. • Dosing: ○ 1600 mg *p.o.* the first day, then 2 × 600 mg *p.o.* next 5 days • Clinical stage: ○ Not defined. • Safety: ○ Adverse effects (most commonly cited): Shock, anaphylaxis, pneumonia, hepatic dysfunction, toxic epidermal necrolysis, Stevens-Johnson syndrome, acute kidney injury, white blood cell count decreased, neutrophil count decreased, platelet count decreased, neurological and psychiatric symptoms, colitis hemorrhagic, supraventricular extrasystoles ○ Contraindications[c]: hypersensitivity to the drug, pregnancy • Interactions[d]
Ribavirin	• Mechanism of action: ○ It is a guanine analogue, which inhibits viral RNA-dependent RNA polymerase. • Dosing: ○ 400 mg q.d., 14 days • Clinical stage: ○ Not recommended as first-line treatment • Safety: ○ Adverse effects (the most commonly cited): Viral and bacterial infection, pharyngitis, respiratory tract infection, skin infection, neoplasm unspecified hemolytic anaemia, leucopenia, neutropenia, thrombocytopenia, lymphadenopathy, hypothyroidism, hyperthyroidism, headache, dizziness, tinnitus, diarrhoea ○ Contraindications[c]: hypersensitivity to the drug, pregnancy, breastfeeding, history of severe pre-existing cardiac disease, hemoglobinopathies • Interactions[d]
Atazanavir	• Mechanism of action: ○ Atazanavir (formerly known as BMS-232632) is an antiretroviral drug of the protease inhibitors (PIs) class. • Dosing: ○ 400 mg q.d., 14 days • Clinical stage: ○ Not defined. • Safety: ○ Adverse effects (most commonly cited): Bloating, chest pain, dark urine, diarrhoea, dizziness, headache, difficulty breathing, vomiting, back pain, cough ○ Contraindications[c]: Hypersensitivity to the drug, severe hepatic insufficiency • Interactions[d]: ○ amiodarone, bepridil, disopyramide, dofetilide, flecainide, quinidine, rifampicin, rifapentine, apixaban, clopidogrel, dabigatran, rivaroxaban, ticagrelor, carbamazepine, phenobarbital, phenytoin, primidone, St John's wort, repaglinide, aliskiren, eplerenone, ivabradine, lercanidipine, ranolazine, bosentan, sildenafil, pimozide, quetiapine, ziprasidone, midazolam (oral), triazolam, cisapiride, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole, domperidone, sirolimus, lovastatin, simvastatin, budesonide, fluticasone, mometasone, triamcinolone
Umifenovir	• Mechanism of action: ○ A viral entry inhibitor of the target cells, with a unique mechanism of action targeting the S protein/ACE2 interaction and inhibiting membrane fusion of the viral envelope. • Dosage: ○ 200 mg *p.o.* , t.i.d., (no more than 10 days) • Clinical stage: ○ Not defined yet. • Safety: ○ Adverse effects (the most commonly cited): allergic reaction as rash ○ Contraindications[c]: Children under 2 years • Interactions: ○ No available data

(Continues)

TABLE 1 (Continued)

Interferon-alpha 2b	• Mechanism of action: ○ It promotes the production of interferon-stimulated genes in infected and neighbouring cells; their products induce an intracellular antimicrobial programme that limits the spread of infectious pathogens. Also, it increases antigen presentation, costimulation, and cytokine production by innate immune cells, leading to enhanced adaptive immune responses. • Dosage: ○ 5 million IU b.i.d. aerosol nebulisation for 5 to 7 days or ○ 3 million IU to 5 million IU *s.c.* , q.d. for 5-7 • Clinical stage: ○ II, III, not recommended as first-line treatment • Safety: ○ Adverse effects (the most commonly cited): Pharyngitis, infection viral, bronchitis, sinusitis, leukopenia, thrombocytopenia, lymphadenopathy, lymphopenia, sarcoidosis, hypothyroidism, hyperthyroidism, anorexia, hypocalcemia, dehydration, hyperuricemia, hyperglycemia, hypertriglyceridemia, dizziness, headache, vision blurred, palpitation, tachycardia, nausea/vomiting, abdominal pain, diarrhoea, alopecia, pruritus, skin dry ○ Contraindications[c]: Hypersensitivity to the active substance or any of the excipients, severe pre-existing cardiac disease, severe renal or hepatic dysfunction, history of autoimmune disease, epilepsy and/or compromised central nervous system (CNS) function, chronic hepatitis with decompensated cirrhosis of the liver, or in patients who are being or have been treated recently with immunosuppressive agents, pre-existing thyroid disease • Interactions[d]: ○ telbivudine, ara-C, cyclophosphamide, doxorubicin, teniposide
Tocilizumab	• Mechanism of action: ○ IL-6 inhibition/reduction in a cytokine storm • Dosage: ○ 8 mg/kg (up to a maximum of 800 mg per dose) per 12 hours • Clinical stage: ○ III • Safety: ○ Adverse effects (most commonly cited): respiratory tract infection, skin infection, hypertension, leucopenia, neutropenia, cough, dyspnea, increase in liver transaminases ○ Contraindications[c]: hypersensitivity to the active substance or any of the excipients, severe infections, severe hepatic dysfunction • Interactions[d]: ○ adalimumab, basiliximab, evolocumab

Abbreviations: b.i.d., twice a day; DRESS syndrome, Drug rash with eosinophilia and systemic symptoms syndrome; ECG, Electrocardiogram; IU, international unit; lapp, referring to Sámi people, indigenous Finno-Ugric people; *p.o.*, *per os*; q.d., once a day; *s.c*, subcutaneously; t.i.d., three times a day.

[a]Mechanism of action of chloroquine and hydroxychloroquine is described in the text below.

[b]Dosage of chloroquine and hydroxychloroquine is described in the text below and Table 2. All listed drugs above require a dose adjustment in renal and hepatic insufficiency.

[c]Numerous special warnings and precautions exist for the use of medications.

[d]Table incudes only drugs that should not be coadministered. Numerous interactions may require a dose adjustment or close monitoring, and with possible interactions likely to be of weak intensity.

3.2.2 | Animal toxicity, pharmacokinetics in animals, and humans and drug safety

Animal toxicity studies compared both drugs in short-term and longer-term assays in five species and by four routes of administration.[53] The overall weighted margin of safety was 2.5/1.0 in favour of hydroxychloroquine, meaning that hydroxychloroquine has 40% toxicity of chloroquine.[53] The tissue levels of chloroquine averaged about 2.5 times those of hydroxychloroquine when an identical dosage regimen of two drugs were given to albino rats.[54] It was apparent that a more rapid and/or extensive transformation of the parent drug took place in rats administered with hydroxychloroquine, and it accounts for virtually all of its lower extent of accumulation in tissues. In human volunteers, in equal doses, two drugs were nearly interchangeable regarding plasma levels.[53] The half-life of both drugs was about 50 hours.[53] Hydroxychloroquine achieved peak plasma concentration after 4 hours, and chloroquine after 5 hours.[53] The absorption of the two drugs, after oral intake, is nearly complete.[53] The difference between drugs exists in the pattern of excretion since hydroxychloroquine excretion in the urine is three times lower and in the feces three times higher than chloroquine.[53,55]

Better hydroxychloroquine long-term clinical safety profile allows a higher daily dosage and fewer drug-drug interactions compared with chloroquine.[42,56] The addition of the hydroxyl molecule makes hydroxychloroquine less permeable to the blood-retinal barrier and allows faster clearance from retinal pigment cells.[57]

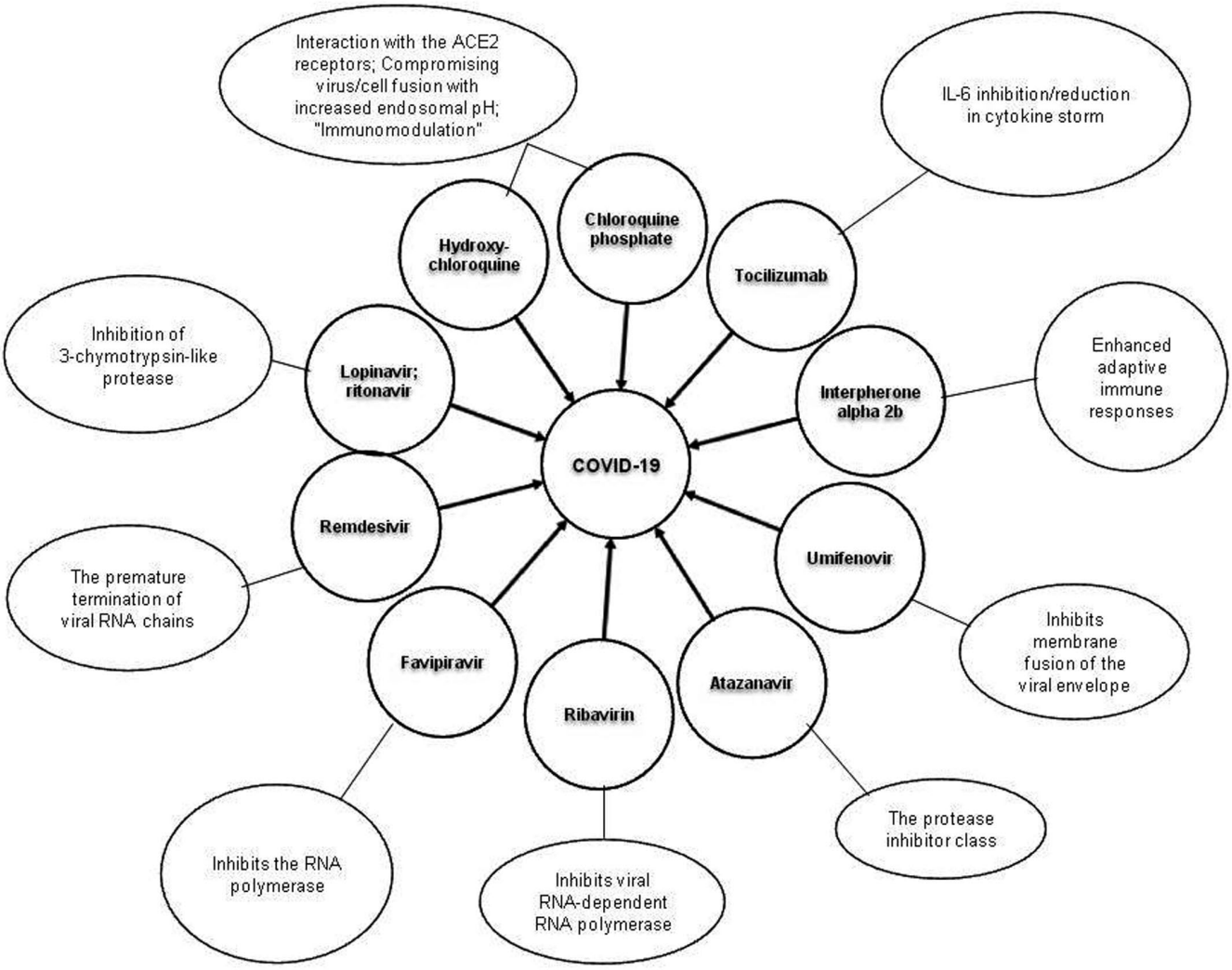

FIGURE 1 Mechanism of action of medications with antiviral activity proposed for the management of COVID-19

Therefore, hydroxychloroquine exhibits lower retinal toxicity in comparison to chloroquine.[42,57] Since the cumulative effect on cardiac conduction was seen in a combination of hydroxychloroquine and azithromycin, electrocardiography monitoring is indicated, and surveillance for QT interval prolongation.[39] Chloroquine and hydroxychloroquine are metabolised by cytochrome P450 (CYP) isoenzymes CYP2C8, CYP2D6, and CYP3A4; therefore, inhibitors and inducers of these enzymes may result in altered pharmacokinetics of these agents.[58]

3.2.3 | Antiviral activity of chloroquine and hydroxychloroquine in clinical trials and current treatment guidelines

Patients with severe COVID-19 are critically ill on mechanical ventilation with multiple organ failure and possible comorbidities. Pharmacokinetic studies are needed to define the optimal dosing regimen in critically ill since pathophysiological changes may modify hydroxychloroquine pharmacokinetic profile.[59] So far, the dosage in critically ill patients is based on population study in patients with rheumatoid arthritis.[59] The recommended hydroxychloroquine dosage is 800 mg once daily, on the first day, that can rapidly reach therapeutic levels in critically ill patients, followed by 200 mg, twice daily for 7 days. These encouraging findings were, to some extent, supported by the results of the exploratory clinical observations, suggesting the superiority of chloroquine vs control to inhibit the exacerbation of COVID-19 pneumonia.[60] The use of chloroquine treatment in more than 100 patients with COVID-19 resulted in improved radiologic findings, enhanced viral clearance, and shortening disease course.[60] However, clinical trial design and outcomes were missing; therefore, the value of the result is questionable.

Another study enrolled 36 COVID-19 positive patients to receive oral hydroxychloroquine sulfate, 200 mg, three times per day, during 10 days, in a hospital setting[61] (Table 2). The primary endpoint was virological clearance at day 6 following inclusion in the study. The secondary endpoints were clinical signs (body temperature,

TABLE 2 Recommendations concerning the posology of chloroquine and hydroxychloroquine in COVID-19 treatment in selected publications

Reference	Dose recommendation	Duration
[62]	CQ 500 mg b.i.d. (CQ 300 mg b.i.d.)	A maximum of 10 days
[62]	CQ base 600 mg starting dose + 300 mg after 12 hrs on day 1, followed by 300 mg b.i.d. from day two to five (per Dutch CDC)	A maximum of 5 days
[62]	CQ 500 mg b.i.d. for 10 days or HCQ 200 mg q.d for 10 days (per Italian Society of Infectious and Tropical disease)	Duration ranges from five to 20 days based on clinical severity
[63]	CQ 500 mg b.i.d.	Seven days
[64]	CQ 500 mg b.i.d.	A minimum of 5 days to a maximum of 10 days
[52]	HCQ 400 mg b.i.d. on day one followed by 200 mg b.i.d. on day two to five	Five days
[61]	HCQ Sulfate 200 mg t.i.d	Ten days
[65]	HCQ 400 mg q.d.	Five days
[66]	HCQ 1200 mg q.d. HCQ 800 mg q.d.	Three days, then 15 days
[67]	CQ 600 mg b.i.d CQ 450 mg b.i.d CQ 450 mg q.d.	Ten days vs One day then 4 days
[68]	HCQ 200 mg b.i.d.	Five days

Abbreviations: b.i.d., twice a day; CDC, Centre for Disease control and prevention; CQ, chloroquine; HCQ, hydroxychloroquine; q.d., once a day; t.i.d., three times a day.

respiratory rate, length of stay at hospital and mortality rate), and the occurrence of side effects.[61] At post-inclusion day 6, 70% of hydroxychloroquine-treated patients were virologically cured, compared with 12.5% in the control group ($P = .001$).[61] Moreover, 100% of patients treated with hydroxychloroquine and azithromycin combination were virologically cured compared with 57.1% in patients treated with hydroxychloroquine.[61] Significant limitations and concerns of drugs cardiotoxicity questioned the safety of this regimen.

In another prospective clinical trial conducted in China, 15 patients were randomised to receive hydroxychloroquine 400 mg per day and standard of care (SOC), for 5 days, or SOC in another 15 patients.[68] On day 7, the authors detected similar virologic clearance between groups, such as 86.7% clearance for hydroxychloroquine plus SOC vs 93.3% in the SOC group.[68] Multicentric, parallel, open-labelled randomised clinical trial included 150 hospitalised adult COVID-19 patients (Table 2).[66] The addition of hydroxychloroquine to the SOC did not increase anti-viral response but accelerated the alleviation of clinical symptoms and recovery of lymphopenia.[66] The efficacy of hydroxychloroquine on the reduction of symptoms (Hazard ratio, 8.83, 95%CI, 1.09 to 71.3) became more evident after post hoc subgroup analysis, which removed the confounding effects of anti-viral agents.[66] However, this study demonstrated no difference in viral clearance between hydroxychloroquine and SOC group against the SOC group, which was the primary outcome.[66]

A randomised, double-blind, phase 2b study aimed to evaluate the safety and efficacy of two chloroquine dosage regimens in 81 patients with severe COVID-19 infection (Table 2).[67] All patients received a combination of ceftriaxone and azithromycin, while 89.6% of the patients received oseltamivir.[67] The primary outcome was mortality 13 days after starting treatment. Mortality was higher in the high-dose arm than in the low-dose arm [death in 16 of 41 patients (39%) vs in 6 of 40 patients (15%) exacerbation; $P = .03$].[67] Moreover, QTcF >500 ms occurred more frequently amongst patients in the high-dose arm (18.9%) compared with the low-dose (11.1%).[67] This study raised concerns at increased mortality rate caused with a combination of high-dose chloroquine (600 mg twice daily), azithromycin and oseltamivir.[67]

In a randomised controlled trial, 62 hospitalised patients with mild COVID-19 pneumonia were randomised to receive hydroxychloroquine 200 mg twice daily for 5 days, plus SOC or SOC.[68] The hydroxychloroquine-treated patients had a 1 day shorter mean duration of fever, and the cough remission time was significantly reduced in that group.[68] None of the hydroxychloroquine treated patients experienced the progression of illness.[68] According to the analysed chest computerised tomography, a more significant proportion of patients had improved pneumonia in the hydroxychloroquine treated group (80.6%, 25 of 31) compared with the control group (54.8%, 17 of 31). Moreover, 61.3% of patients in the former group had a significant pneumonia resolution. While adverse events occurred amongst two (6.4%) of the hydroxychloroquine treated patients, none of the patients in the control group experienced them.[68]

TABLE 3 Summary of studies reporting the use of hydroxychloroquine or chloroquine for COVID-19 treatment

Reference	Country	
[66]	China	• Drug: Hydroxychloroquine (HCQ) • Design: Multicentric, open-label, randomised controlled clinical trial • Number of patients: 150 • The primary outcome: the primary endpoint was the 28-day negative conversion rate of SARS-CoV-2 • The secondary outcome: negative conversion rate at day 4, 7, 10, 14 or 21, the improvement rate of clinical symptoms within 28-day, normalisation of C-reactive protein, and blood lymphocyte count within 28-days. • Findings: HCQ did not result in a higher negative conversion rate but more alleviation of clinical symptoms than SOC alone in patients hospitalised with COVID-19 without receiving antiviral treatment • Study limitations: n/a
[67]	Brazilian Amazon	• Drug: chloroquine (CQ) • Design: parallel, double-blinded, randomised, phase IIb clinical trial • Number of patients: 81 • The primary outcome: reduction in lethality by at least 50% in the high-dosage group • The secondary outcome: clinical status, laboratory examinations, and electrocardiogram results • Findings: the higher CQ dosage not recommendable for critically ill patients with COVID-19 • Study limitations: small sample size; single-centre design; lack of placebo control group; the absence of exclusion criteria based on the QTc interval at baseline
[68]	China	• Drug: HCQ • Design: randomised, parallel-group clinical trial • Number of patients: 62 • The primary outcome: time to clinical recovery and clinical characteristics, pulmonary recovery • The secondary outcome: n/a • Findings: There was no significant difference in the age and sex distribution between the two groups of patients, but there are are significant differences in time to clinical recovery between the two groups. • Study limitations: N.A.
[59]	France	• Drug: HCQ • Design: prospective cohort study • Number of pts: 13 • The primary outcome: n/a • The secondary outcome: n/a • Findings: some of the dosing regimens will fail to reach therapeutic levels, while others will probably induce levels higher than 2 mg/L • Study limitations: n/a
[65]	China	• Drug: HCQ • Design: Randomised clinical trial • Number of pts: 30 • The primary outcome: negative conversion rate of SARS-CoV-2 nucleic acid in respiratory pharyngeal swab on day 7 after randomisation • The secondary outcome: n/a • Findings: n/a • Study limitations: small sample size
[61]	France	• Drug: HCQ • Design: Open-label non-randomised study • Number of pts: 36 • The primary outcome: virological clearance at day-6 post-inclusion • The secondary outcome: virological clearance overtime during the study period, clinical follow-up and occurrence of side effects. • Findings: 100% of patients treated with HCQ and azithromycin combination were virologically cured comparing with 57.1% in patients treated with HCQ only • Study limitations: small sample size
[80]	United States of America	• Drug: HCQ • Design: Observational Study • Number of pts: 1446 • The primary outcome: the time from study baseline to intubation or death • The secondary outcome: n/a • Findings: there was no significant association between HCQ use and incidence of the need for intubation or death • Study limitations: missing data for some variables and potential for inaccuracies in the electronic health records, such as lack of documentation of smoking and coexisting illness for some patients; the single-centre design

(Continues)

TABLE 3 (Continued)

Reference	Country	
[81]	United States of America	• Drug: HCQ • Design: a randomised, double-blind, placebo-controlled clinical trial • Number of pts: 821 • Primary outcome: the incidence of either laboratory-confirmed COVID-19 or illness compatible with COVID-19 within 14 days • Secondary outcome: n/a • Findings: high doses of HCQ did not prevent illness compatible with COVID-19 when initiated within 4 days after a high-risk or moderate-risk exposure • Study limitations: the lack of availability of diagnostic testing

Abbreviations: CQ, chloroquine; HCQ, hydroxychloroquine; n/a, not available.

However, the methodological limitations of this study preclude firm evidence of hydroxychloroquine efficacy and safety (Table 3).

Differences exist in chloroquine and hydroxychloroquine dosage regimen between national guidelines. National Health Commission of the People's Republic of China issued "Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19" and recommended dosage and duration of chloroquine treatment (Table 2).[52] The suggested course of chloroquine treatment ranges from a minimum of 5 days to a maximum of 10 days (Table 2).[52,63] Dutch Centre for Disease Control proposed chloroquine regimen in adults for up to 5 days, while guidelines by the Italian Society of Infectious and Tropical disease (Lombardy section) recommended 10 days (Table 2).[62] Kapoor et al. suggested a dosage regimen of hydroxychloroquine for up to 7 days (Table 2).[63]

Chloroquine and hydroxychloroquine therapy were suggested for the treatment of a broad spectrum of COVID-19 severity ranging from mild respiratory symptoms in patients with comorbidities to severe respiratory failure.[62] However, according to Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with COVID-19, hydroxychloroquine/chloroquine should only be used in the context of a clinical trial because of the knowledge gap.[39] The same applies to the combination of hydroxychloroquine/chloroquine and azithromycin.[39] This is supported by the statement of the US National Institute of Health "The COVID-19 Treatment Guidelines" that insufficient clinical data prevent for or against the recommendation of chloroquine or hydroxychloroquine for the treatment of COVID-19 (Rating of Recommendation grade/Levels of evidence –AIII).[69] The same body recommends against using high-dose chloroquine (600 mg twice daily for 10 days) for the treatment of COVID-19 (AI).[69]

Since studies have documented serious dysrhythmias in patients with COVID-19 treated with chloroquine or hydroxychloroquine, especially in combination with azitromycine, authorities of the FDA strongly recommend use of chloroquine or hydroxychloroquine in hospital settings and for the propose of clinical trials.[70] When chloroquine or hydroxychloroquine is used, patients should be monitored for adverse effects, especially prolonged QTc interval (A, III).[71] European Medicines Agency (EMA) also considers the use of chloroquine and hydroxychloroquine only in clinical trials or emergency programmes.[71] Outside clinical trials, it should be prescribed per nationally established protocols. The United Kingdom Commission on Human Medicines (CHM) advises ministers on the safety, efficacy, and quality of medicinal products released the same recommendation as EMA.[69] The latest issue of Interim clinical guidelines for adults with suspected or confirmed COVID-19 in Belgium states that it was decided not to recommend hydroxychloroquine off-label use for COVID-19 in this country anymore, except within ongoing clinical registered trials after careful reassessment of the study-related risk/benefit.[40]

Significant concerns were raised after the publication of the analysis of extensive multinational registry 96 000 admitted COVID-19 patients, the vast majority with mild disease, including about 15,000 exposed to chloroquine or hydroxychloroquine, alone or in combination with macrolide.[72] This study did not find any benefit in the groups treated with these drugs, after adjustment, and even found increased mortality and higher frequency of ventricular arrhythmia. These data attracted so much attention that the Executive group of Solidarity Trial, an international clinical trial dedicated to an effective treatment search for COVID-19, launched by the WHO and partners, temporarily paused the hydroxychloroquine use, during the safety data review by the Data Safety Monitoring Board.[73] Then, suddenly because of the unavailability to provide sufficient data to the third party peer review, which raised a question about data accuracy and validity, the authors retracted the article.[72] This added even more confusion in the medical community in an already stressful situation as doctors worldwide are struggling to save the lives of millions of patients affected with COVID-19.

The Data Monitoring Committee of the Randomised Evaluation of COVID-19 thERapY (RECOVERY) Trial on hydroxychloroquine, stated that the trial would continue recruitment since mortality reported in the analysis by Mehra were not consistent with those observed in the RECOVERY trial.[74] RECOVERY Trial is a large, randomised trial of treatments for patients admitted to hospitals with COVID-19.[75] Over 10 000 patients have been randomised to the following arms: Lopinavir-Ritonavir, Low-dose Dexamethasone, Hydroxychloroquine, Azithromycin, and Tocilizumab, or no additional treatment.[74] Moreover, on Tuesday 27th May, French public health agency advised against using hydroxychloroquine outside of clinical trials.[76] Shortly after that, the national medicines regulator suspended its use in clinical trials because of the safety concerns.[76,77]

Recently, the National Institutes of Health (NIH) stopped study known as ORCHID (The Outcomes Related to COVID-19 treated with hydroxychloroquine amongst In-patients with symptomatic Disease study).[78] NIH based the decision on results that showed no beneficial effects of hydroxychloroquine for the treatment of COVID-19 in hospitalised patients.[78]

Chloroquine and hydroxychloroquine use for COVID-19 treatment are controversial. The results from the double-blind, randomised clinical trials, including a large number of participants, will add better insight into the role of these two drugs as already available and affordable antimalarial therapy.

The risk of serious adverse events associated with chloroquine and hydroxychloroquine was recently reanalysed within the pharmacovigilance data from the EudraVigilance Database of the EMA.[40] Across Europe, 182 cases of QTc prolongation have been reported with hydroxychloroquine since the beginning of the epidemic, mostly if used in high dosages and/or in combination with the antibiotic azithromycin or other drugs known to prolong the QTc interval.[40] Up to date, the QTc-prolonging potential of chloroquine was estimated after a single drug dose since its different posology in malaria treatment.[42] The first study involving a large number of patients,[79] which examined the QTc-prolonging potential of chloroquine in the context of COVID-19 treatment, indicated that this drug could significantly prolong the QTc interval in a clinically relevant manner.[79] In this study, the QTc interval was measured after multiple doses of chloroquine during 48 hours.[79] Since chloroquine has a substantial volume of distribution, and long half-life, the QTc interval prolongation risk persists for days after the discontinuation of therapy for a prolonged period.

4 | THE ETHICAL ISSUE ON EMERGENCY USE OF CHLOROQUINE AND HYDROXYCHLOROQUINE

Based on the above-discussed data, the ethical question is whether the administration of chloroquine and hydroxychloroquine in the settings of COVID-19 is experimental and requires ethical committee approval or an off-label position ethically justifiable as the best available treatment despite its registration status. The current epidemiological situation justifies the prioritisation of ethical review of study proposals for fast track institutional ethical review. Namely, according to WHO guidance, the use of experimental interventions under the emergency use in infectious disease outbreak is referred to as "monitored emergency use of unregistered and experimental interventions" (MEURI).[82] Experimental interventions on an emergency basis outside clinical trials should be provided in several situations such as nonexistent proven effective treatment; inability to start clinical studies immediately; data support the efficacy and safety of the drug, at least from laboratory or animal studies.[82] Additionally, an appropriately qualified scientific committee can suggest drugs use outside clinical trials approved by relevant country authorities or suitably qualified ethical committees.[82] In the countries where clinical trials are conducted, it is a moral obligation to refer patients to the hospital with an active clinical trial on chloroquine/hydroxychloroquine for COVID-19 treatment.[83]

Potentially, the increased use of chloroquine/hydroxychloroquine can cause a shortage on the market and inaccessibility for standard indications as the treatment of autoimmune disease.[71] To prevent unnecessary strain on supply chains, patients should only receive their regular supply of drugs prescribed by their medical doctor. Also, each national drug agency should ensure sufficient quantities of these drugs, primarily if no clinical trials are conducted in a country during an outbreak.

5 | CONCLUSIONS

There is rationale from pre-clinical evidence of chloroquine and hydroxychloroquine effectiveness and their safety from long-time clinical use in other indications to justify clinical research of these drugs in patients with COVID-19. However, these drugs have been used in small randomised trials, case series and clinical trials with conflicting study reports. Therefore, data from high-quality, coordinated, multicentric clinical trials are urgently needed to establish the position of chloroquine and hydroxychloroquine in COVID-19 infection treatment. The ethical issue on emergency use of chloroquine and hydroxychloroquine should be carefully managed, with adherence to the MEURI framework or be ethically approved as a trial, as stated by the WHO.

DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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