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Chloroquine and hydroxychloroquine in the treatment of malaria and repurposing in treating COVID-19

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ABSTRACT

Chloroquine (CQ) and Hydroxychloroquine (HCQ) have been commonly used for the treatment and prevention of malaria, and the treatment of autoimmune diseases for several decades. As their new mechanisms of actions are identified in recent years, CQ and HCQ have wider therapeutic applications, one of which is to treat viral infectious diseases. Since the pandemic of the coronavirus disease 2019 (COVID-19), CQ and HCQ have been subjected to a number of *in vitro* and *in vivo* tests, and their therapeutic prospects for COVID-19 have been proposed. In this article, the applications and mechanisms of action of CQ and HCQ in their conventional fields of anti-malaria and anti-rheumatism, as well as their repurposing prospects in anti-virus are reviewed. The current trials and future potential of CQ and HCQ in combating COVID-19 are discussed.

Contents

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1. Introduction

Quinine, which is obtained from the bark of cinchona trees, was first discovered in the 1600s and used as a major therapy for malaria until the 1920s (Achan et al., 2011). During World War II, due to insufficient availability of quinine under wartime pressure, synthetic drugs derived from quinine were inspired. Chloroquine (CQ) was first synthesized as an anti-malarial drug candidate by a German scientist Hans Andersag in 1934 (Frosch, Schmitt, Bringmann, Kiefer, & Popp, 2007). However, chloroquine was not adopted for medical use on a large scale due to its toxicity until it was re-synthesized by an American company and tested to be proved with efficacy and safety in the early 1940s (Thomé, Lopes, Costa, & Verinaud, 2013). By introducing a hydroxyl group into CQ, hydroxychloroquine (HCQ) was synthesized in 1946, and it was approved for medical use as an alternative to CQ because of its favorable efficacy and reduced toxicity in 1955 (Browning, 2014). Since then, both CQ and HCQ have been used broadly for the prevention and treatment of malaria. Besides, the anti-malarial effects, the anti-rheumatic property of anti-malarial drugs was discovered during World War II from the observation that the soldiers taken prophylactic CQ for malaria gained improvement in autoimmune-induced rashes and inflammatory arthritis (Md Abdul Alim Al-Bari, 2015; Ben-Zvi et al., 2012). In the past seven decades, CQ and HCQ have been commonly used to treat patients with rheumatologic disorders like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (Schrezenmeier & Dörner, 2020).

Despite CQ and HCQ are being gradually restricted for the treatment of malaria due to the emergence of plasmodium strains resistant to chloroquine and the cross-resistant to CQ analogs (Haldar, Bhattacharjee, & Safeukui, 2018; Warhurst, Steele, Adagu, Craig, & Cullander, 2003), the repurposing potentials of CQ and HCQ in treating many other diseases have been reported in recent years. It has been shown that CQ and HCQ could have beneficial effects in preventing thrombosis (Petri, 2011; Ruiz-Irastorza et al., 2006), reducing the risk of cardiovascular disease in RA patients (D. Liu et al., 2018), treating neoplastic diseases (Plantone & Koudriavtseva, 2018), and combating bacterial and viral infectious diseases other than malaria (Raoult et al., 1999; Savarino, Boelaert, Cassone, Majori, & Cauda, 2003a). In particular, the anti-viral effect of CQ and HCQ has gained much attention since the pandemic of coronavirus disease 2019 (COVID-19). In this article, we review the applications and mechanisms of action of CQ and HCQ in their conventional fields of anti-malaria and anti-rheumatism, as well as their repurposing prospects in anti-virus. Furthermore, the current trials and future potential of CQ and HCQ in combating COVID-19 are discussed.

2. Pharmacokinetics

After oral administration of CQ or HCQ, the gastrointestinal absorption is fast and sufficient, and the blood concentration reached the peak value 2–3 h after dosing (Fan et al., 2015). The bioavailability of CQ and HCQ is 70%–80% (Browning, 2014). CQ can penetrate into most tissues with a large distribution volume of about 65,000 L and the final elimination half-life 40–60 days (AlKadi, 2007). In contrast, HCQ can accumulate and maintain a steady-state concentration in the body tissues for 4–6 months (Tett, Cutler, & Day, 1992). Both CQ and HCQ undergo biotransformation in the liver by enzymes CYP3A4, CYP2D6, CYP2C8, or CYP1A1. (Gil & Gil Berglund, 2007; K.-A. Kim, Park, Lee, & Lim, 2003; Leden, 1982; Li, Björkman, Andersson, Gustafsson, & Masimirembwa, 2003; Projean, Morin, Tu, & Ducharme, 2003; Walker & Iyun, 1984). The main metabolites of CQ through the N-dealkylation reaction include desethylchloroquine, which retains the anti-malarial activity, and bis-desethylchloroquine (Leden, 1982; Walker & Iyun, 1984). HCQ is transformed into deacetylated metabolites in the liver. CQ is primarily eliminated in the urine as more than half of the dose recovered unchanged in the urine, 10% of the dose recovered in the urine as its desethyl-metabolite, and about 8% of chloroquine is excreted in feces or milk (Ducharme & Farinotti, 1996). HCQ is mainly eliminated by the kidney, and the other part is excreted by feces and skin (Munster et al., 2002). As CQ and HCQ can be stored in visceral tissue while most of them are metabolized in the liver and excreted slowly, a long-lasting antimalarial activity is expected.

3. CQ and HCQ in the treatment of malaria

3.1. Etiology of malaria

Malaria is one of the most prevalent infectious diseases affecting human beings. The World Malaria Report 2019 from the World Health Organization (WHO) estimates 228 million diagnosed cases and 0.4 million deaths of malaria globally in 2018. Malaria mostly affects developing regions of tropical and subtropical areas, particularly African. Young children under 5-year old and pregnant women are most vulnerable due to their compromised immune systems (World Health Organization, 2019). Therefore, malaria remains a severe public health problem worldwide. Malaria is caused by infection of *Plasmodium* parasites. The infective *Plasmodium* parasites are transmitted to humans by the bite of female Anopheles mosquitoes (Mace, Arguin, & Tan, 2018). The malaria parasite sporozoites inoculated to human by mosquito infect liver cells, mature and rupture hepatocytes to release merozoites (human liver stage), which further attack red blood cells (RBCs) for asexual multiplication and cause rupture of RBCs by releasing merozoites (human blood stage), leading to clinical symptoms (Baker, 2010). Because of the presence of parasites in RBCs during blood stage in infected individuals, malaria can also be transmitted to the newborns *via* maternal-neonatal transmission (Hartman, Rogerson, & Fischer, 2010) and be passed from person to person through blood transfusion (Kitchen & Chiodini, 2006), organ transplant (Menichetti et al., 2006), or the shared use of blood-contaminated needles (Alavi, Alavi, & Jaafari, 2010; Chau et al., 2002). There are five species of *Plasmodium* identified to be pathogens causing human malaria: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* (Antinori, Galimberti, Milazzo, & Corbellino, 2012). Among them, *P. falciparum and P. vivax* are the predominant species with the highest incidences, particularly *P. falciparum* causes severe malaria (World Health Organization, 2019).

3.2. Anti-malarial activities of CQ and HCQ

With the advantages of high efficacy, good tolerability and low-cost, CQ and HCQ were the first-line treatments for malaria for several decades. However, the extensive use of CQ and HCQ resulted in the emergence of CQ-resistant *P. falciparum* strains within 20 years after the introduction of CQ (Harinasuta, Suntharasamai, & Viravan, 1965). The CQ resistance of *P. falciparum* has been widely spread in most malaria-endemic areas. As artemisinin was discovered by the Nobel Prize laureate Youyou Tu's Team, artemisinin and its active analogs have been increasingly applied in the treatment of malaria because of their rapid action, high efficacy to CQ-resistant plasmodium strains and the ability to prevent malaria transmission (Abay, 2013; Tu, 2011). The recommended treatment for *falciparum* and CQ-resistant *vivax* malaria has been changed to artemisinin-based combination therapy (ACT) (World Health Organization, 2019). Although cases of *P. vivax* resistant to CQ have been reported (Plantone & Koudriavtseva, 2018), *P. vivax* remains sensitive to CQ in some vivax malaria-endemic regions, where chloroquine is still broadly used.

According to the Treatment of Malaria Guideline from WHO and Center of Disease Control and Prevention (CDC), CQ and HCQ are used for the treatment of CQ susceptible uncomplicated *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* malaria, and for chemoprophylaxis against relapse in *P. vivax* or *P. ovale* malaria in pregnant or lactating women and travelers (Centers for Disease Control and Prevention & World

Health Organization, 2015; World Health Organization, 2015). The suggested dose for CQ is total 25 mg base /kg body weight (bw) given in three consecutive days (10, 10 and 5 mg base/kg bw, respectively) to treat primary blood infection (World Health Organization, 2015), and a weekly dose of total 300 mg base is suggested for prophylaxis purpose(Styka, et al., 2020). Three-day CQ dose combined with 14-day primaquine (PQ) dose (0.25–0.75 mg/kg bw per day) is recommended for primary treatment as well as relapse prevention (World Health Organization, 2015). It is important to note that patients' G6PD (glucose-6-phosphate dehydrogenase) activity should be tested before treatment initiation to adjust the dosage of PQ and avoid acute hemolytic anemia triggered by PQ in G6PD deficiency patients (World Health Organization, 2016).

CQ monotherapy or CQ combined with PQ is the first-line policy for fighting *P. vivax* in parts of the Americas, South-East Asia, Western Pacific regions, and Eastern Mediterranean regions (World Health Organization, 2019). Therapy efficacy tests have been conducted to confirm high efficacy of CQ or CQ-PQ in Bhutan (Wangchuk, et al., 2016), Colombia (Mesa-Echeverry, Niebles-Bolívar, & Tobón-Castaño, 2019), India (Rishikesh et al., 2015; Saravu et al., 2016), Thailand (Chu et al., 2018), Iran (Azarian et al., 2018), Pakistan (Waqar, Khushdil, & Haque, 2016), China-Myanmar border (Hui Liu et al., 2014), and Cambodia (Amaratunga et al., 2014). Only a few countries in Africa are with endemic of *vivax* malaria. It has been reported high a failure rate for CQ treatment in Ethiopia (Getachew et al., 2015), however, in Mauritania the efficacy of CQ retained 100% as reported in 2015 (Ould Ahmedou Salem et al., 2015). In addition, CQ is the first-line treatment for *falciparum* malaria in Guatemala, Haiti, Honduras, and Nicaragua (World Health Organization, 2019). High CQ efficacy and low incidence of CQ resistance-related mutations in *P. falciparum* were reported in Haiti (Neuberger, Zhong, Kain, & Schwartz, 2012), Honduras (Torres et al., 2013) and Nicaragua (Sridaran, Rodriguez, Soto, De Oliveira, & Udhayakumar, 2014).

3.3. Anti-malarial mechanisms of CQ and HCQ

The precise anti-malarial mechanism of action from CQ and HCQ has not been completely clarified. The major theory of the anti-malarial mechanism is related to the blockade of the detoxification process in the *Plasmodium* parasites (Thomé et al., 2013). At the blood stage when the *Plasmodium* invades the RBCs, the plasmodium ingests hemoglobin from the RBC cytosol into the food vacuole and decomposes hemoglobin to obtains amino acids proteins synthesis, which is necessary for their growth (Mohandas & An, 2012). The toxic oxidized heme consisting of ferriprotoporphyrin IX (FPIX) hematin is generated from proteolytic degradation of hemoglobin, which is transformed into nontoxic crystalized polymers named hemozoin by heme polymerase of the parasite (Chou & Fitch, 1992). As the food vacuole is a lysosomal isolated acidic compartment, the weak alkalinity of CQ could help it diffuse across the vacuole membrane, where CQ turns to protonated form that is not able to diffuse out (Krogstad & Schlesinger, 1986). Different mechanisms of CQ inhibiting the detoxification of FPIX have been found. The accumulated CQ in the food vacuole could inhibit the polymerization of FPIX by complexing with FPIX, which is highly toxic inducing parasite cell lysis (Fitch et al., 1982). Sullivan et al. found that the complex of CQ and hematin could cap the growing hemozoin polymer to stop further polymerization, resulting in building up of toxic hematin and damaging the parasite (D. J. Sullivan, Gluzman, Russell, & Goldberg, 1996). The enzymes involved in the formation of hemozoin may also be considered as targets of CQ. The histidine-rich protein-2 of *P. falciparum* (Pfhrp-2) has been found to mediate the formation of hemozoin by binding to FPIX (Sullivan Jr., Gluzman, & Goldberg, 1996). It was reported that CQ, though did not bind to Pfhrp2 directly, could displace FPIX from Pfhrp2 and form CQ-FPIX complex, thereby exerting toxicity to the parasite cells (Pandey et al., 2001).

Another hypothesis suggested that the target of CQ is not in the lysosome but the nucleus. It was proposed that CQ could interact or directly bind to DNA and RNA causing disruption of replication and transcription process, which could lead to inhibition of growth and reproduction, or induction of apoptosis of parasite cells (Li, 2006). Also, there are arguments regarding the mechanism of CQ uptake. Instead of diffusion, an importing transporter was suggested to be involved in CQ accumulation in parasite cells. Sanchez et al. demonstrated that a Na^+/H^+ exchanger (NHE) on the cytoplasmic membrane may serve as a CQ importer carrying CQ and Na^+ into the cells by an exchange of protons. This hypothesis was supported by the observation of inhibition of CQ uptake by NHE inhibitors (Sanchez, Wünsch, & Lanzer, 1997).

With a similar structure, HCQ may have identical anti-malarial mechanisms to CQ (FDA/CDER, 2017). The mechanism of anti-malarial activities of CQ and HCQ is still controversial. It may be beneficial to further illuminate the mechanism and identify novel targets for overcoming CQ/HCQ resistance.

3.4. Adverse effects

CQ and HCQ are known to be well tolerated in the conventional drug delivery scheme for malaria treatment and show good safety in treating pregnant women (Diav-Citrin, Blyakhman, Shechtman, & Ornoy, 2013; Sciascia et al., 2016). However, due to the narrow therapeutic window of CQ, adverse reactions could be severe in clinical application. Compared to CQ, HCQ has a hydroxyl group, which reduces its toxicity while retaining the original CQ effect (Ben-Zvi, Kivity, Langevitz, & Shoenfeld, 2012). The common adverse reactions of CQ/HCQ, including the gastrointestinal tract and skin adverse reactions, are usually mild and disappear rapidly after discontinuation, thus not likely to affect the continuity of treatment (Bahloul et al., 2017; Joyce, Fabre, & Mahon, 2013). The major CQ/HCQ-induced adverse reactions in the gastrointestinal tract are vomiting, nausea, stomachache, diarrhea, anorexia, and weight loss, usually at the early stage of medication (Kelley et al., 2014; Quach et al., 2017). Side effects on the skin include skin rash, pruritus, and hair loss (Ajayi, 2019; Soria et al., 2015). Rare cases with severe side effects of renal failure and allergy reactions have been reported (Tönnesmann, Kandolf, & Lewalter, 2013). Moreover, consideration of reduced dose should be taken for patients with liver disorders and alcoholic liver diseases since about half of oral administrated CQ/HCQ is metabolized in the liver (Tönnesmann et al., 2013).

CQ/HCQ can also cause serious and even fatal adverse reactions in heart and retina, epilepsy, extrapyramidal symptom, hypoglycemia, as well as poisoning caused by overdose (H. Liu et al., 2015; Yogasundaram, Hung, Paterson, Sergi, & Oudit, 2018). The irreversible toxic retinopathy developed after CQ/HCQ treatment is one of the main adverse effects. It has been reported that the incidence of retinopathy can reach 1% after continuous use of CQ for 5– 7 years (Tehrani, Ostrowski, Hariman, & Jay, 2008). The mechanism underlying CQ/HCQ-induced retinopathy is the binding of CQ/HCQ to melanin, particularly in the pigment cells at the epithelial cell layer of the eye, and destroy rod cells and cone cells (Melles & Marmor, 2014; Michaelides, Stover, Francis, & Weleber, 2011). Therefore, clinical monitoring during CQ/HCQ treatment is essential for the prevention of retinopathy (Tehrani et al., 2008).

The data of clinical studies from a large number of samples showed that the incidence of these adverse reactions of CQ/HCQ was low and generally mild or moderate (Marmor, Carr, Easterbrook, Farjo, & Mieler, 2002; Sato, Mano, Iwata, & Toda, 2020). As the efficacy of CQ/HCQ has been clinically certified, it is safer to use it under the strict control of indications and contraindications.

3.5. Emergence and mechanisms of CQ resistance in malaria

In 1957, the first case of CQ-resistant *falciparum* malaria was reported on the Thai-Cambodian border. Since then, the *P. falciparum*

strains resistant to CQ spread from Southeast Asia and South America to Africa and has been found in nearly all endemic areas (Khatoon, Baliraine, Bonizzoni, Malik, & Yan, 2009; Mita, Tanabe, & Kita, 2009; Restrepo-Pineda, Arango, Maestre, Do Rosário, & Cravo, 2008). The emergence and spread of malaria resistance seriously hampered malaria control and became the main cause of the sharp rise in malaria incidence worldwide. As a result, CQ and HCQ are no longer effective in most *falciparum* malaria prevalent areas and are mainly used to treat CQ susceptible *Plasmodium vivax* (Nzila, 2006). Besides, there is an increasing trend of CQ-resistance in *P. vivax* in recent years. There have been reports regarding the emergence of *P. vivax* CQ-resistance in some countries and regions that use CQ or CQ-PQ as first-line malaria treatment, such as Bolivia (Añez et al., 2015), Brazilian Amazon (Marques et al., 2014), and the northeastern region of Myanmar (Yuan et al., 2015).

The drug resistance mechanisms of CQ and HCQ may be the result of multiple factors (Coppée, Sabbagh, & Clain, 2020). In CQ-resistant *P. falciparum*, the variation of two proteins may be related to their resistance (Le Bras & Durand, 2003; Valderramos & Fidock, 2006). One is the transporter protein P-glycoprotein (P-gp/ABCB1) homolog 1 (Pgh1) on the membrane of vacuoles of the parasite, which is encoded by a multidrug resistance (MDR) gene, the *Plasmodium falciparum* multidrug resistance-1 (*pfmdr1*) gene on chromosome 5 (Skrzypek & Callaghan, 2017). As Pgh1 is a homolog of the ATP- binding cassette (ABC) transporter P-gp, which contributes to the cross-cytomembrane transport of substrates out of cells (Kathawala, Gupta, Ashby Jr., & Chen, 2015), Pgh1 may have a similar function pumping CQ and CQ analogs from the cytoplasm to the vacuoles (Anderson et al., 2005; Jovel, Björkman, Roper, Mårtensson, & Ursing, 2017; Tang, Ye, Huang, & Zheng, 2020). Under the pressure of CQ/HCQ, the *pfmdr1* gene has many geographical mutations to gain resistance (Skrzypek & Callaghan, 2017). The typical two mutations are the replacement of tyrosine with histidine at codon 191 (Y191H) and the replacement of alanine with serine at codon 437 (A437S) (Briolant et al., 2010), resulting in the loss of CQ uptake function of the expression product Pgh1 on the acid food vacuolar membrane and the decrease of CQ concentration in the vacuoles (Osman, Mockenhaupt, Bienzle, Elbashir, & Giha, 2007; Reed, Saliba, Caruana, Kirk, & Cowman, 2000). The other protein related to CQ resistance is the membrane-located *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) encoded by the CG-2 gene on chromosome 7 and *pfcrt* gene adjacent to CG-2 gene. PfCRT is a member of the drug metabolite transporter (DMT) system, which is also situated on the *Plasmodium* food vacuole membranes, participating in drug competition for hemoglobin (Chinappi, Via, Marcatili, & Tramontano, 2010; Fidock et al., 2000). Similar to Pgh1, PfCRT acquire the resistance to CQ by mutation. However, opposite to Pgh1, it decreases CQ concentration in the vacuoles by accelerating CQ efflux (Anderson et al., 2005). At least 32 types of mutations of this gene facilitate the resistance to CQ, and the most important one is K76T where lysine at codon 76 is replaced by threonine (Lakshmanan et al., 2005). The surprising and severe influence of this mutation is that the sensitivity to CQ could not recover and the mutated *P. falciparum* remained highly resistant even after 5-year alternative treatment with ACT (Chatterjee et al., 2016). Besides, the mutation of these two transporters, the increased activity of some enzymes to strengthen the detoxification of FPIX could also induce the resistance of CQ/HCQ (J. Kim et al., 2019).

3.6. CQ analogs and combination therapy as strategies to overcome drug resistance

It has been found that a variety of compounds without fixed chemical properties can restore the sensitivity to CQ (de Souza et al., 2019). These compounds, when co-administrated with CQ, were observed to increase the sensitivity of some CQ resistant *P. falciparum* strains *in vitro* (Ch'ng et al., 2013; Deane, Summers, Lehane, Martin, & Barrow, 2014; Edaye, Tazoo, Bohle, & Georges, 2015; Kashyap et al., 2018). The major mechanism involved is the inhibition of the active drug efflux pump *pfcrt* (van Schalkwyk & Egan, 2006). The CQ combination approaches in the past two decades are listed in Table 1. Although CQ resistant reversal agents are not yet applicable in the clinical practice for CQ/HCQ resistant *falciparum* malaria, great breakthroughs may be made in the research of other reversal agents in the future.

4. CQ and HCQ in the treatment of autoimmune diseases

Autoimmune diseases including systemic sclerosis, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren's syndrome, are linked with substantial morbidity and mortality (Gabriel & Michaud, 2009; Goldblatt & O'Neill, 2013). CQ and HCQ have been applied to the treatment of RA and SLE for more than 70 years (Schrezenmeier & Dörner, 2020). It was first found that anti-malaria drugs can be used to treat skin diseases when Payne applied quinine to patients with SLE (Smith & Cyr, 1988). Since then, strong evidence showed that treatment with CQ or HCQ resulted in a significant improvement of RA as well as SLE (Bjelle, Björnham, Larsen, & Mjörndal, 1983; Durcan, O'Dwyer, & Petri, 2019). Both CQ and HCQ are able to increase the long-term survival of patients, while HCQ is associated with fewer adverse effects (Marmor, 2004). Therefore, HCQ has become a more frequent option when treating rheumatic diseases compared to CQ. Although neither CQ nor HCQ went through conventional drug development, they have been recommended in current treatment guidelines for RA (Smolen et al., 2017) and SLE (Fanouriakis et al., 2020).

Several mechanisms of action are postulated to explain how CQ/HCQ works in treating autoimmune rheumatic diseases. One mechanism

Table 1
Development of CQ derivatives and combination treatments targeting CQ resistance.

Therapy	Status	Proposed mechanism
Chloroquine + primaquine (Bray et al., 2005)	Phase 3 Completed	Reverse the resistance by inhibit the transport of chloroquine *via* PfCRT
Chloroquine + Verapamil (Martin et al., 2009)	*In vivo*	
Chloroquine + mibefradil (Ch'ng et al., 2013)	*In vitro*	
Chloroquine + Chlorpheniramine (Deane et al., 2014)	*In vitro*	
Chloroquine + diltiazem (Menezes et al., 2003)	*In vitro*	Unclear mechanism with a much higher susceptibility of Brazilian strains
Chloroquine + dehydroepiandrosterone (Safeukui et al., 2004)	Phase 3 Suspended	Through induction of protective immune response
Chloroquine + chlorpromazine (Kalkanidis, Klonis, Tschan, Deady, & Tilley, 2004)	*In vitro*	Magnify antimalarial effect by binding FPIX to inhibit hemozoin formation
Chloroquine + 4-amino-7-chloroquinoline (Kalkanidis et al., 2004)	*In vivo*	Interact with PfCRT and indirectly enhance the accumulation of CQ by modifying the proton gradient
Chloroquine +10-N-substituted acridones (Kelly et al., 2007)	*In vitro*	Specifically target K76T of PfCRT to reverse the resistance
Chloroquine + tetrandrine (Ye, Van Dyke, & Rossan, 2013)	*In vivo*	Stimulate Pgh1 ATPase to cause depletion of parasite ATP thereby limiting the energy of the pump; Block the transcription factor that controls the production of the pump
Chloroquine + 3-iodo-chloroquine (Edaye et al., 2015)	*In vitro*	Bypass PfCRT-mediated resistance
Dextran nanoparticles bearing chloroquine diphosphate (CHQ-DEX-NPs) (Kashyap et al., 2018)	*In vitro*	The submicron particle size and the affinity to the structure of the DEX vector will facilitate the phagocytosis of CHQ-DEX-NPs (increase drug uptake)

suggests CQ/HCQ can affect the activity of lysosomes and autophagosomes. Lysosomes and autophagosomes are involved in antigen processing, presentation, and autoimmune activation (Ghislat & Lawrence, 2018; Münz, 2016). It was widely believed that the basic side chain of CQ/HCQ allows drug accumulation in lysosomes and causes a substantial increase in the intra-lysosomal pH, leading to impairment of maturation of lysosomes and autophagosomes, thereby inhibiting the autoimmune activation (Ohkuma & Poole, 1978). Recent studies suggest that CQ/HCQ can modulate the lysosomal function mainly by blocking lysosome-autophagosome fusion (Mauthe et al., 2018; Sundelin & Terman, 2002). It is suggested that other mechanisms are involved in the anti-rheumatic effect, such as inhibiting proinflammatory cytokine expression (Willis et al., 2012) and/or Toll-like receptor signaling et al. (Chen, Szodoray, & Zeher, 2016).

5. Anti-viral activities of CQ and HCQ

Although CQ was first developed to treat malaria, the focus has largely moved from anti-malaria to anti-rheumatic, and the anti-virus effect. The anti-viral effect of CQ has been extensively evaluated in the last two decades.

5.1. Anti-viral mechanisms of CQ and HCQ

The anti-viral effect of CQ is mainly attributed to its alkalinity. As previously mentioned, CQ can cause alkalization within acidic organelles, including lysosomes and endosomes (Ohkuma & Poole, 1978), thereby two mechanisms may explain its anti-viral activity. First, most viruses require low pH for fusion, penetration, or uncoating during infection. In addition, the endosomal pathway is critical for viruses to replicate and infect the host (Sieczkarski & Whittaker, 2002). Since CQ prevents endosomal acidification, the elevated pH level results in inhibition of lysosome functions, thereby inhibiting the activity of viruses such as influenza B virus (Shibata et al., 1983) and hepatitis A virus (M. A. A. Al-Bari, 2017). Second, CQ may inhibit the post-translational modification of the virus envelope glycoprotein by interfering with the activity of specific enzymes that require low pH (Chiang et al., 1996; Rolain, Colson, & Raoult, 2007). Another mechanism may be attributed to the immunomodulatory effect of CQ. Recent studies suggested that CQ can inhibit the production of IFN-γ, TNF-α, and neopterin in cell culture models, which cytokines are critical for filoviruses infection (Akpovwa, 2016).

5.2. Anti-viral applications of CQ and HCQ

Using CQ/HCQ as anti-HIV treatment has been extensively studied. It has been well-established that CQ/HCQ can inhibit HIV replication *in vitro*. The main mechanism may be the inhibition of post-translational protein modification in T cells and monocytes (Naarding, Baan, Pollakis, & Paxton, 2007). At high concentrations, CQ/HCQ showed preventive effect prior to HIV infection, while the curative effect was demonstrated with low concentrations of CQ/HCQ in HIV-infected cells. Moreover, the anti-HIV effect of CQ/HCQ has been demonstrated in several clinical studies. A randomized, double-blind, placebo-controlled clinical trial was carried out to investigate the anti-HIV-1 effect of HCQ in 40 asymptomatic patients (Sperber et al., 1995). The patients were assigned to either HCQ (800 mg/day) group or placebo group for 8 weeks of treatment. The data showed that treatment with HCQ was able to decrease the virus load compared to the placebo group. The recoverable HIV-1 RNA level decreased significantly in the HCQ group, while increased in the placebo group. Besides, reductions in cultured virus, interleukin-6 level, as well as stabilization of immune function were demonstrated in the HCQ treatment group after 8 weeks of treatment. The study suggests that HCQ may be useful to combat HIV-1 infection. In another 16-week clinical trial, the anti-HIV effect of HCQ (800 mg/day) was investigated along with zidovudine (500 mg/day) (Sperber et al., 1997). In accordance with the initial trial, HCQ showed a significant effect in reducing HIV-1 RNA levels. Moreover, while the zidovudine group showed a trend of drug resistance after 8 weeks of treatment, no patient in the HCQ group showed increased HIV-1 RNA levels or cultured virus throughout the trial. Notably, no significant adverse effect was observed with HCQ treatment in both trials. Altogether, these two trials suggested that HCQ may be a potential option for treating HIV-1 infected patients. One study indicated that CQ can hinder immune activation, therefore may benefit certain groups of HIV-infected patients (Murray et al., 2010). In this study, the patients were subjected to CQ (250 mg/day or 500 mg/day) or placebo for 2 months. The memory T cells population following CQ treatment showed a median decreased of 2.5%, with no significant change observed in the placebo group. Besides, it is demonstrated that Ki-67 expression and plasma LPS level were decreased after 1 month of CQ administration, suggesting a reduced immune activation. However, no decrease in HIV RNA levels was observed in the trial, indicating 250 mg of CQ may be insufficient to decrease virus load. Other trials exploring the potential of using HCQ as part of the combinational treatment also gained promising results (N. I. Paton & Aboulhab, 2005). However, the drug has not been recommended as an option of HIV treatment and it warrants further investigation.

CQ/HCQ also has anti-viral effects against the Zika virus, influenza viruses, hepatitis viruses, and others. Delvecchio et al. reported that CQ can suppress ZIKV infection in different cell models (Delvecchio et al., 2016). Shiryaev et al. showed that CQ can limit ZIKV vertical transmission in pregnant mouse model, suggesting CQ might be a candidate for the treatment and prophylaxis of ZIKV (Shiryaev et al., 2017). *In vitro* studies reported that CQ was able to inhibit influenza A replication in a pH-dependent manner (Di Trani et al., 2007; Ooi, Chew, Loh, & Chua, 2006). Clinical studies suggested that CQ treatment may safely reduce the risk of relapse in patients with autoimmune hepatitis (Mucenic, Mello, & Cançado, 2005; Raquel Benedita Terrabuio et al., 2019). In the recent trial, patients were randomized to receive CQ (250 mg/d), or placebo for 36 months. A significant difference in relapse-free survival was demonstrated between the CQ and placebo group (59.3% and 19.9%, respectively, hazard ratio, 2.4; 95% CI, 1.05–5.5; $P = 0.039$). Besides, the adverse effects were moderate and can be controlled with symptomatic medication. Small scale pilot trials indicated that CQ might improve the symptoms of chronic hepatitis C patients (Helal, Gad, Abd-Ellah, & Eid, 2016; Peymani et al., 2016). In the pilot study investigating the anti-HCV effect of CQ, a significant decrease of plasma ALT and HCV RNA levels were observed in patients receiving 150 mg/d CQ treatment compared to the placebo group after 8 weeks of treatment. However, an increase in virus load was observed within 1 month after treatment cessation. A similar anti-HCV effect was demonstrated in a clinical trial investigating HCQ as part of a combinational treatment. The results showed that the triple therapy (HCQ with pegylated interferon plus ribavirin) had a higher percentage of early virological response than the standard treatment (pegylated interferon plus ribavirin). The improved response may be due to the antiviral effect of HCQ, which then augmented the inhibitory activity of the standard treatment. The triple therapy was also shown to be well-tolerated with mild adverse effects similar to the standard treatment.

Not all the encouraging *in vitro* studies could be translated into *in vivo* settings. For instance, Paton et al. demonstrated the result of a clinical trial that CQ showed no effect in preventing infection (Nicholas I. Paton et al., 2011). There are several possible explanations for the failure of translating *in vitro* effects into clinical outcomes, including the narrow therapeutic index, poor drug penetration in a specific compartment, and selectivity against a different strain of influenza A viruses (Di Trani et al., 2007; Savarino, 2011). Another case of interest is the chikungunya virus. Although CQ exhibited great anti-viral effect *in vitro* (Delogu & de Lamballerie, 2011), animal studies showed that it actually enhanced alphavirus replication (Maheshwari, Srikantan, & Bhartiya, 1991; Roques et al., 2018). It is believed that the deleterious

effect is due to the immunomodulatory and anti-inflammatory effects of CQ (Katz & Russell, 2011; Savarino et al., 2003). Also, a clinical trial conducted in 2006 suggested that CQ has no beneficial effect on the course of disease progression in patients with chikungunya virus (De Lamballerie et al., 2008).

5.3. CQ and HCQ in the treatment of coronavirus infections

The recent focus has largely shifted to the anti-Cov effect of CQ/HCQ due to the COVID-19 pandemic. Keyaerts et al. reported that CQ is able to inhibit HCoV-OC43 activity both *in vitro* and *in vivo* (Keyaerts et al., 2009). Kono et al. reported that CQ can suppress the viral replication of HCoV-229E by affecting the MAPKs signaling pathway (Kono et al., 2008). In 2004, CQ was found to have a significant inhibitory effect on SARS-Cov infection (Keyaerts, Vijgen, Maes, Neyts, & Van Ranst, 2004; Vincent et al., 2005). It is postulated that the mechanisms of action are though hindering the terminal glycosylation of angiotensin-converting enzyme-2 (ACE2), a functional receptor of SARS-CoV spike protein (Li et al., 2003). In addition, CQ/HCQ may also inhibit the biosynthesis of sialic acids, which are components of receptors of SARS-CoV (Savarino, Di Trani, Donatelli, Cauda, & Cassone, 2006).

6. Updates of CQ and HCQ in the treatment of COVID-19

6.1. Current statue of CQ and HCQ in combating COVID-19

As of June 30th, 2020, the COVID-19 has led to more than 10,273,001 confirmed cases and 505,295 deaths worldwide (www.ecdc.europa.eu/). So far, a tremendous effort has been made globally to search for efficacious drugs against COVID-19, and among all these potential candidates, anti-malarial drugs CQ and HCQ have gained considerable attention.

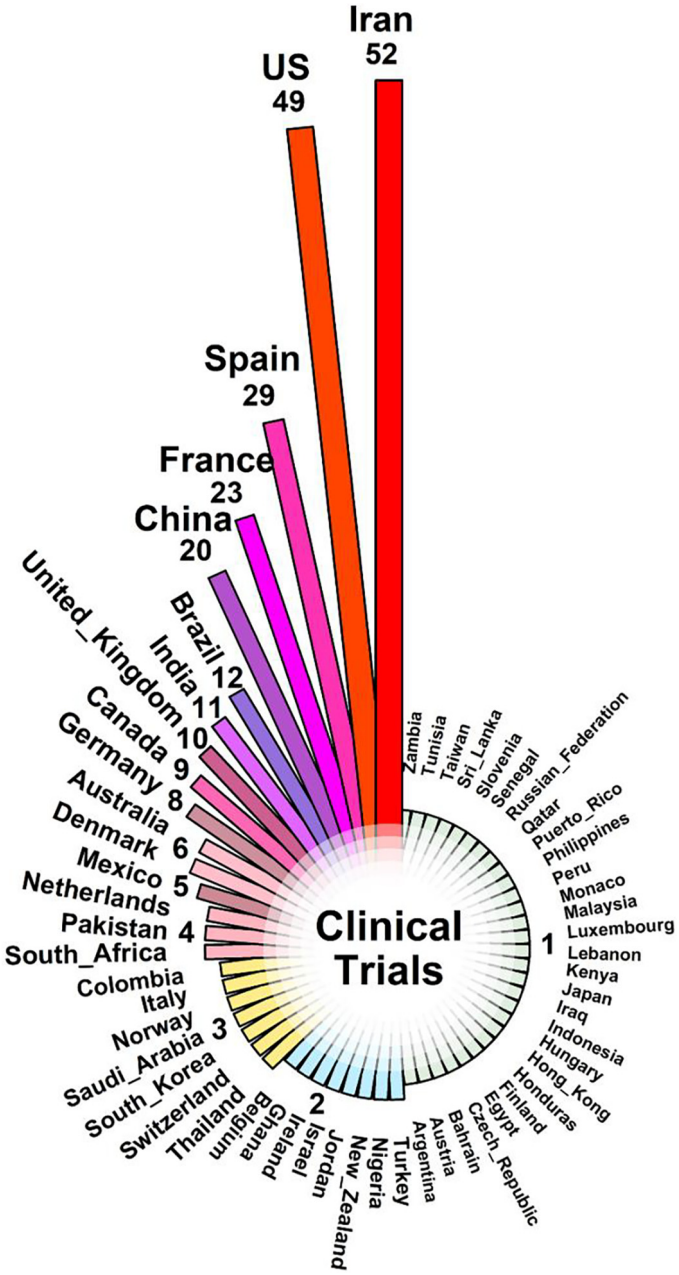

Fig. 1. The number of CQ/HCQ related clinical trials registered in each country from January to May 2020.

Table 2
List of Clinical Studies Involving CQ/HCQ as Treatment for COVID-19.

Reference	Country	Period (2020)	Participants	Treatment	Adverse Events	ICU Patients	Mortality	Outcome	Summary
(Yu et al., 2020)	China	02/01-04/08	568 Control: 520 HCQ: 48	HCQ (200 mg bid[a], 7–10 days)	NA[b]	520/48	238/9	Among the critically ill patients, mortalities were 18.8% in HCQ group and 45.8% in control group.	The usage of HCQ significantly decreased the mortality rate in critically ill COVID-19 patients.
(Z. Chen, et al., 2020)	China	02/04-02/28	62 Control: 31 HCQ: 31	HCQ (200 mg bid, 5 days)	0/2	4/0	0	At Day 6 post inclusion, 80.6% of patients in HCQ treatment group showed improved pneumonia in comparison with control group (54.8%).	The use of HCQ significantly shortened the time to clinical recovery.
(J. Chen, et al., 2020)	China	02/06-02/25	30 Control: 15 HCQ: 15	HCQ (400 mg qd[c])	4/3	0	0	At Day 4 post inclusion,86.7% of patients in HCQ treatment group converted to COVID-19 negative; 93.3% of patients in control group converted to COVID-19 negative.	No significant difference was found in HCQ treated moderate COVID-19 patients in comparison with patients in control group.
(Huang et al., 2020)	China	02/07-03/08	373 Control: 176 HCQ: 197	CQ (500 mg qd or bid)	57/53	0/0	0/0	At Day 14 post inclusion, 91.7% of patients in CQ group had undetectable viral RNA in comparison with 51.7% of patients in control group.	The results indicated CQ could be effective in COVID-19 treatment.
(W. Tang, et al., 2020)	China	02/11-02/29	150 Control: 75 HCQ: 75	HCQ (1200 mg/day for 3 days and 800 mg/day for the remaining days of total 2–3 weeks)	7/21	NA	NA	At Day 28 post inclusion, 85.4% of patients from HCQ group converted to COVID-19 negative, in comparison to 81.3% of patients in control group.	The usage of HCQ did not result in a higher negative-conversion rate in COVID-19 patients.
(Lee et al., 2020)	South Korea	02/21-03/21	72 LPV/r[d]: 45 HCQ: 27	LPV/r (400 mg/100 mg/12-h) HCQ (400 mg/24-h)	2/1	NA	2/0	18% of the patients in LPV/r group had disease progression, which was lower than that of patients in HCQ group (44%).	The results indicated that LPV/r was more effective in preventing the progression of COVID-19.
(Gautret et al., 2020a)	France	03/01-03/16	36 Control: 16 HCQ only: 14 HCQ + AZ: 6	HCQ (200 mg tid[e], 10 days) AZ (500 mg on day 1 followed by 250 mg/day for the next four days)	NA	0	0	At Day 6 post inclusion, 70% of patients in HCQ-treated group converted to COVID-19 negative; 12.5% of patients in control group converted to COVID-19 negative.	HCQ treatment is significantly associated with viral load reduction, and AZ accelerated this process.
(Mallat et al., 2020)	USA	03/01-03/25	34 Non-HCQ: 11 HCQ: 23	HCQ (400 mg bid for day 1 followed by 400 mg qd for 10 days)	NA	0/0	0/0	At Day 14 post inclusion, 47.8% of patients converted COVID-19 negative, compared to 90.9% of patients in control group.	COVID-19 patients require a longer recovery time with the usage of HCQ.
(Novales et al., 2020)	Spain	03/01-03/25	166 Non-HCQ: 43 HCQ: 123	HCQ (a loading dose of 800 mg + 400 mg, followed by a maintenance dose of 400 mg/day)	NA	NA	21/27	22% of patients in HCQ group died, in comparison with 48.8% in control group; 21.1% of patients in HCQ group were transferred to "Hospital Hotel", comparing to 7% in control group; 56.9% of patients in HCQ group were discharged, comparing to 44.2% in control group.	HCQ increased the cumulative mean survival of COVID-19 patients.
(Gautret et al., 2020b)	France	03/03-03/21	80(NA)	HCQ (200 mg tid[e], 10 days) + AZ (500 mg on day 1 followed by 250 mg/day for the next four days)	7	3	1	At Day 14 post inclusion, 81.3% (65/80) were discharged from hospital.	A beneficial effect of HCQ + AZ combination in COVID-19 treatment was suggested
(Million et al., 2020)	France	03/03-03/31	1061	HCQ (200 mg tid[e], 10 days) AZ (500 mg on day 1 followed by 250 mg/day for the next four days)	25	38	8	Among 1061 patients with HCQ treatment, 973 patients had good clinical outcomes and virological cures, 46 patients had poor clinical outcomes (were transferred to ICU or died)	The combination of HCQ + AZ was safe and associated with low fatality in COVID-19 patients.
(Magagnoli et al., 2020)	USA	03/09-04/11	368 Non-HCQ: 158 HCQ: 97 HCQ + AZ: 113	Details NA	NA	NA	18/27/25	27.8%, 22.1%, and 11.4% of deaths in HCQ, HCQ + AZ, and no HCQ groups, respectively; 13.3%, 6.9%, and 14.1% of mechanical ventilations in HC, HC + AZ and no HCQ groups, respectively.	No evidence of the usage of HCQ, either with or without AZ, could reduce the risks of deaths or mechanical ventilations in patients with COVID-19 was found.
(Rosenberg et al., 2020)	USA	03/15-03/28	1061 HCQ + AZ: 735 HCQ: 271 AZ: 211	NA	NA	226/ 52/ 23/ 27	189/ 54/ 21/ 28	71.6%, 73.8%, 89.6%, and 84.1% of patients from HCQ + AZ, HCQ only, AZ only, and neither drug treatment groups were discharged from hospital.	HCQ, AZ, or both treatments were not significantly associated with mortality.

(continued on next page)

Table 2 (*continued*)

Reference	Country	Period (2020)	Participants	Treatment	Adverse Events	ICU Patients	Mortality	Outcome	Summary
			Neither: 221						
(Mahevas et al., 2020)	France	03/17-03/31	181 Non-HCQ: 97 HCQ: 84	HCQ (600 mg/day)	0/8	17/13	4/3	20.2% of patients in HCQ group were transferred to ICU or died, comparing to 22.1% in non-HCQ group.	The results did not support that the usage of HCQ could help COVID-19 patients.
(Ahmad, Alam, Saadi, Mahmud, & Saadi, 2020)	USA	03/19-03/30	54	HCQ (200 mg tid for 7 days or 400 mg bid for day 1 then 400 mg qd for 6 days)+ DOXY (100 mg bid for 7 days)	2	6	3	At Day 7 post inclusion, 85% of patients showed clinical recovery.	The combination of DOXY-HCQ may improve the clinical outcomes of COVID-19 patients.
(Borba et al., 2020)	Brazil	03/23-NA	81 Low CQ:41 High CQ:40	CQ Low:450 mg bid for day 1 then 450 mg qd for 4 days High: 600 mg bid for 10 days	NA	NA	4/7	At Day 13 post inclusion, the mortality rate is 27%, and a trend toward higher lethality was found in High CQ group in comparison with patients in Low CQ group	High CQ dosage should not be recommended in COVID19 treatment

[a] bid: bis in die, twice a day
[b] NA: Not applicable or not available
[c] qd: quaque die, once a day
[d] LPV/r: Lopinavir/ritonavir
[e] tid: ter in die, three times a day

On February 18th, 2020, CQ was added to the "Guideline on diagnosis and treatment of COVID-19 (Trail 6th edition)" issued by National Health Commission (NHC) of China (National Health Commission of China, 2020); on March 28th, 2020, an emergency use authorization for use of CQ and HCQ in the treatment of COVID-19 was issued by the Food and Drug Administration (FDA) of United States (US Food and Drug Administration, 2020); on April 1st, 2020, a limited usage notification was issued by European Medicines Agency (EMA) contending that CQ and HCQ should only be used in clinical trials or emergency use programs (European Medicines Agency, 2020).

Some *in vitro* data supporting the anti-COVID19 effect of CQ and/or HCQ has been published or preprinted. Wang et al. revealed that the combination of remdesivir and CQ was effective against COVID-19 in Vero E6 cells (Wang et al., 2020). Liu et al. showed that HCQ could inhibit the infection of SARS-CoV-2 viruses in Vero E6 cells and might be a better option due to its less toxicity and anti-inflammatory function (J. Liu et al., 2020). It is also found that the antiviral ability of HCQ was more potent than that of CQ in Vero cells (Yao et al., 2020). Weston et al. tested the antiviral ability of 20 FDA approved drugs in Vero cells and found that CQ and HCQ could both reduce mRNA levels and replication process of SARS-CoV-2 (Weston et al., 2020). Touret et al. screened a chemical library containing 1520 approved drugs and identified 90 compounds with anti-COVID-19 activity and CQ/HCQ among the top hits (Touret et al., 2020).

Since CQ and HCQ have shown anti- COVID-19 activities *in vitro*, the potential effect of CQ/HCQ in the treatment and/or prevention of COVID-19 infection has attracted attention globally. As of June 14th, a total of 3138 COVID-19 clinical trials have been registered in more than 60 countries (Data retrieved from http://covid19.trialstracker.net/). Among them, CQ and/or HCQ were mentioned as intervention methods in 297 trials, which represented 9.5% of all clinical trials. Among all the countries, Iran had the highest number of CQ/HCQ related clinical trials (52), followed by US (49), Spain (29), France (23), and China (20). The rest of the countries have a trial number ranging from 1 to 12, as detailed in Fig. 1. Over 330,000 patients were enrolled in these CQ/HCQ trails.

A number of clinical studies have been conducted to evaluate the efficacy of CQ and HCQ in the treatment of COVID-19, as listed in Table 2. Huang et al. explored the effectiveness of CQ on 373 COVID-19 patients and found that CQ treatment accelerated the process of viral elimination (Huang et al., 2020), however, the dosage of CQ should be carefully considered because higher CQ dosage might be associated with higher lethality (Borba et al., 2020). HCQ treatment is often combined with AZ (Azithromycin) or DOXY (Doxycycline), and several clinical studies have suggested the effectiveness of HCQ treatment in COVID-19 patients. Yu et al. showed that the usage of HCQ significantly decreased the mortality rate in critically ill COVID-19 patients (Yu, Wang, & Li, 2020). Chen et al. reported that the involvement of HCQ significantly shortened the clinical recovery time of COVID-19 patients (Chen et al., 2020), which was also confirmed in another clinical trial study in Spain (de Novales et al., 2020). Gautret et al. showed that HCQ treatment was significantly associated with viral load reduction, and AZ could accelerate this process (Gautret et al., 2020a; Gautret et al., 2020b). The effectiveness of HCQ + AZ or HCQ + DOXY combinations in COVID-19 treatment was also supported in two other clinical studies (Ahmad, Alam, Saadi, Mahmud, & Saadi, 2020; Million et al., 2020).

Aside from these supportive studies, there is evidence opposing the effectiveness of CQ and HCQ in the prevention and treatment of COVID-19. Despite CQ and HCQ can block SARS-CoV-2 infection in kidney epithelial Vero E3 cells, this effect appears to be cell type-specific. Hoffman et al. reported that CQ and HCQ did not inhibit the entry of SARS-CoV-2 virus into lung cells *in vitro*, as CQ and HCQ did not target to pH-independent transmembrane serine protease TMPRSS2, which is a key molecule for viral infection in airway epithelial cells (Hoffmann et al., 2020). Moreover, many clinical studies have shown non-significant or even worse results in HCQ treatment groups. Chen et al. found that there was no significant difference in HCQ treated COVID-19 patients in comparison with patients in the control group (Chen et al., 2020). Tang et al. found that the usage of HCQ did not result in a higher negative-conversion rate in COVID-19 patients (Tang et al., 2020). A similar result was reported by Mahévas et al. showing the usage of HCQ did not help COVID-19 patients in another clinical study (Mahevas et al., 2020). Magagnoli et al. and Rosenberg et al. reported that HCQ + AZ treatment was not significantly associated with the mortality of COVID-19 patients (Magagnoli et al., 2020; Rosenberg et al., 2020). In one study carried out by Mallat et al., COVID-19 patients with HCQ treatment required a longer recovery time in comparison with patients in the control group (Mallat et al., 2020). As more evidence showed that CQ and HCQ have limited benefit to the recovery of COVID-19 patients and are unlikely to effectively reduce mortality,

the usage of CQ and HCQ in treating COVID-19 became restrained. On June 15th, 2020, the US FDA has revoked the emergency use authorization of CQ and HCQ to treat hospitalized COVID-19 patients (US Food & Drug Administration, 2020). The potential adverse effects on the cardiovascular system other serious side effects observed in CQ/HCQ-treated patients are considered to outweigh the benefits of CQ and HCQ in treating COVID-19.

6.2. Potential mechanisms of CQ and HCQ acting on COVID-19

The typical process of viral infection usually involves the following steps: endocytosis of viral particles; transport and uncoating leading to the release of the viral genome; transcription/translation/post-translational modification of viral proteins and assembly followed by exocytosis. The possible mechanisms of CQ/HCQ in the treatment and prevention of COVID-19 may be related to inhibiting these steps.

The infection process of COVID-19 is mediated through the interaction of spike (S) protein on virus and ACE2 on host cells (Yan et al., 2020). CQ has been reported with the ability to inhibit glycosylation of the ACE2 receptor, which directly affects the spread of SARS-CoV infection in host cells (Vincent et al., 2005). Moreover, recently *in silico* simulation showed that CQ/HCQ could prevent the access of S proteins to host cell surface ACE2 proteins by interacting with S proteins (Fantini, Di Scala, Chahinian, & Yahi, 2020). Therefore, inhibiting the interaction between S protein and ACE-2 might partially explain the prevention process. Besides, it has been demonstrated that CQ suppresses the expression of phosphatidylinositol binding clathrin assembly protein (PICALM), further affecting clathrin-mediated endocytosis of nanoparticles (Pelt et al., 2018). COVID-19 falls in the size and shapes of nanoparticles, and the general decrease of endocytosis ability by CQ/HCQ might also contribute to COVID-19 prevention (Hu, Frieman, & Wolfram, 2020).

After entry into host cells, coronavirus utilizes trypsin-like proteases in the lysosome to cleave the surface S proteins and facilitate the fusion with lysosome in a pH-dependent manner. CQ is a weak base molecule that accumulates in the acidic organelles such as lysosomes, leading to a change of their acidification status (Savarino et al., 2003a). The elevated pH in lysosomes may inhibit the enzymatic activity of lysosome proteases and hence the infection process.

The inhibition of the autophagic process by CQ/HCQ might also account for the effects of COVID-19 prevention. The viral replication process occurs in the intermediate compartment of the endoplasmic reticulum and Golgi complex, which is directly linked to autophagosome biogenesis (Ujike & Taguchi, 2015). After CQ/HCQ treatment, the elevated pH in lysosomes inhibits the autophagic process, which might affect the replication process of COVID-19 (Bonam,

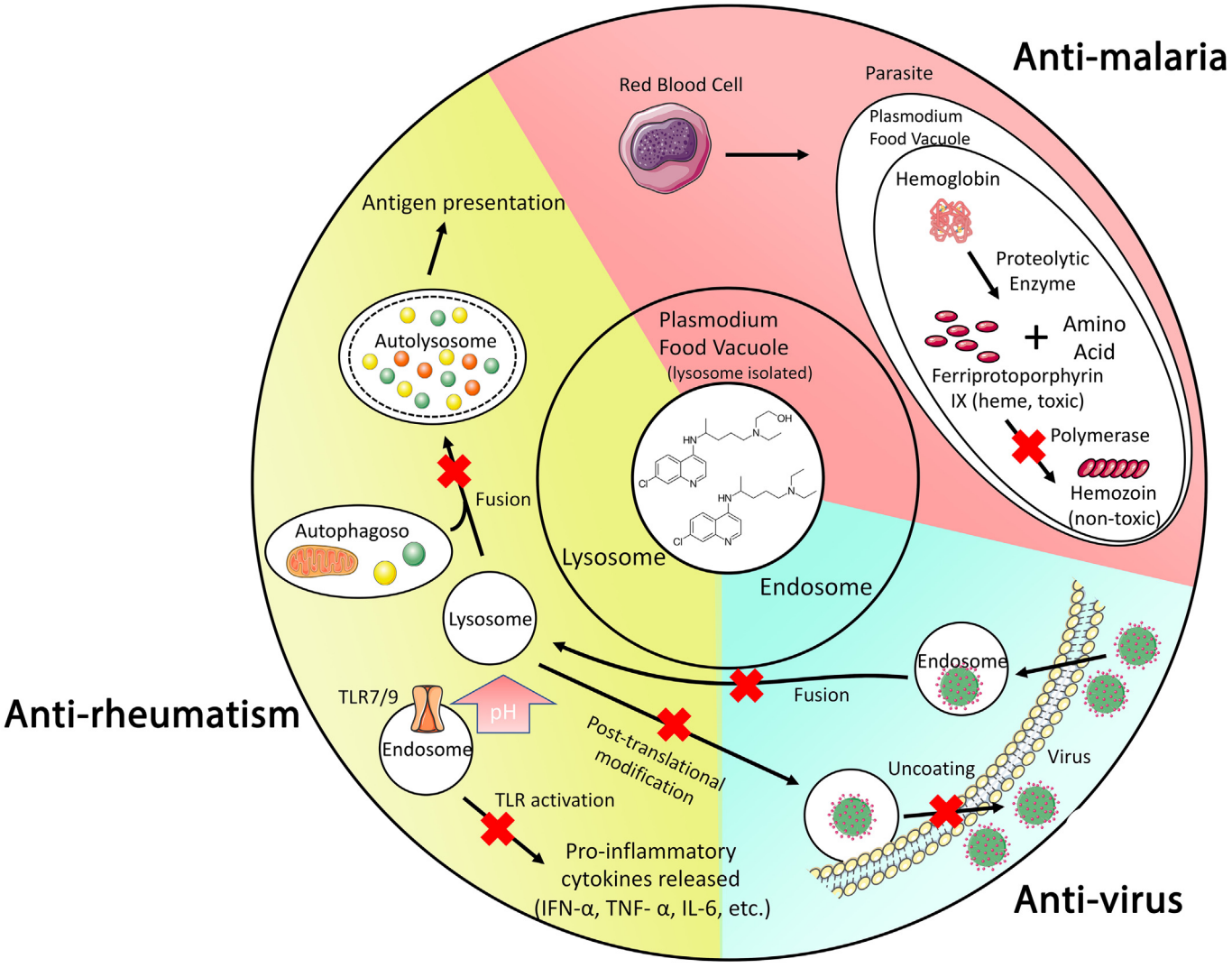

Fig. 2. Schematic summary of anti-malarial, anti-viral, and anti-rheumatic mechanisms of action of CQ and HCQ. The biological processes where CQ/HCQ has inhibitory effects are marked with red crosses. The pH elevating effect of CQ/HCQ in lysosome and endosome is represented by a pink up arrow next to the lysosome and endosome.

Muller, Bayry, & Klionsky, 2020). Besides, the recycled materials accompanying the autophagic process might be utilized in the nucleation process of COVID-19, and the inhibition of autophagic process could also halt the replication process, and hence prevent the process of viral infection.

7. Conclusion

CQ and HCQ have been used as anti-malarial drugs and anti-rheumatic drugs for a long period. While the continuous emergence and spread of resistant *Plasmodium* parasite strains have restricted the use of CQ and HCQ in the treatment of malaria, these drugs show repurposing potential in treating various diseases. In particular, the anti-viral effects of CQ and HCQ have become a research "hotspot" due to the current pandemic of COVID-19. As summarized in Fig. 2, no matter for conventional use in malaria and rheumatic diseases, or for repurposing application in anti-virus, the weak alkalinity of CQ/HCQ plays a critical role in targeting acidic organelles like lysosome and endosome to exert multiple effects. However, cell type-specificity should be taken into consideration as the therapeutic target can be altered in different types of cells. The effectiveness of CQ and HCQ may be limited when pH-insensitive molecules like TMPRSS2 play critical roles in COVID-19 viral infection for specific cell types. The efficacy of CQ and HCQ in COVID-19 treatment remains controversial and reliable reports from large scale double-blinded randomized trials are still limited. Also, considering the long half-life of CQ and HCQ, prolonged follow-up of CQ/HCQ-treated COVID-19 patients is necessary to access long-term side effects. Studies with improved design and risk-benefit assessment are urgently required regarding the usage of CQ and HCQ in treating COVID-19.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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