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Review article

Hydroxychloroquine can potentially interfere with immune function in COVID-19 patients: Mechanisms and insights

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ABSTRACT

The recent global pandemic due to COVID-19 is caused by a type of coronavirus, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). Despite rigorous efforts worldwide to control the spread and human to human transmission of this virus, incidence and death due to COVID-19 continue to rise. Several drugs have been tested for treatment of COVID-19, including hydroxychloroquine. While a number of studies have shown that hydroxychloroquine can prolong QT interval, potentially increasing risk of ventricular arrhythmias and Torsade de Pointes, its effects on immune cell function have not been extensively examined. In the current review, an overview of coronaviruses, viral entry and pathogenicity, immunity upon coronavirus infection, and current therapy options for COVID-19 are briefly discussed. Further based on preclinical studies, we provide evidences that i) hydroxychloroquine impairs autophagy, which leads to accumulation of damaged/oxidized cytoplasmic constituents and interferes with cellular homeostasis, ii) this impaired autophagy in part reduces antigen processing and presentation to immune cells and iii) inhibition of endosome-lysosome system acidification by hydroxychloroquine not only impairs the phagocytosis process, but also potentially alters pulmonary surfactant in the lungs. Therefore, it is likely that hydroxychloroquine treatment may in fact impair host immunity in response to SARS-CoV-2, especially in elderly patients or those with co-morbidities. Further, this review provides a rationale for developing and selecting antiviral drugs and includes a brief review of traditional strategies combined with new drugs to combat COVID-19.

1. Introduction

Wuhan Municipal Health Commission (WMHC) reported 27 cases of viral pneumonia on 12th December 2019. These patients had a recent history of exposure to wildlife animals at the Huanan Seafood Wholesale Market in Wuhan, China, where poultry, snake, and bats were sold. On January 7, 2020, the etiological agent of a novel pneumonia was identified as a severe acute respiratory syndrome coronavirus (SARS-CoV-2) [1,2]. In March 2020 the World Health Organization (WHO) declared the novel coronavirus outbreak a global pandemic, as this virus rapidly spread to other countries and was named as coronavirus disease, 2019 (COVID-19) [3]. Currently, there are no proven antiviral medications/therapies available for COVID-19, and treatment guidelines for COVID-19 vary between countries. The WHO guidelines are, in general, recommending supportive care with management of symptoms, and advise caution with pregnant women, pediatric patients, and patients who have significant co-morbidities. In the current review, an overview of coronaviruses, viral entry and pathogenicity, immunity upon coronavirus infection, and current therapeutic options for COVID-19 are briefly discussed. We provide preclinical evidence suggesting that hydroxychloroquine (HCQ)/chloroquine (CQ) interferes with host immune function and impacts the *in vivo* immune response to SARS-CoV-2, especially in the elderly and patients with co-morbidities, providing insight as to its lack of clinical efficacy against SARS-CoV-2 [4], despite promising *ex vivo data*.

2. Overview of coronaviruses

Coronaviruses (CoVs) are the largest group of viruses belonging to the Nidovirales order and Coronaviridae family. Coronaviridae is further

Abbreviations: TLR, Toll-like receptor; IL, interleukin; CQ, Chloroquine; HCQ, Hydroxychloroquine; ACE2, Angiotensin-converting enzyme 2; MHC, Major histocompatibility complex.

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divided into two subfamilies, Coronavirinae and Torovirinae. The alpha, beta, gamma, and delta coronaviruses belong to Coronavirinae. There are seven coronaviruses that infect humans and have been identified since the mid-1960s. They consist of 1) 229E (alpha coronavirus), 2) NL63 (alpha coronavirus), 3) OC43 (beta coronavirus), 4) HKU1 (beta coronavirus), 5) MERS-CoV (the beta coronavirus), 6) SARS-CoV (the beta coronavirus) and 7) SARS-CoV2 (the beta coronavirus COVID-19) [5–7]. Fig. 1 depicts the coronavirus structure and genome. Coronaviruses contain an RNA genome with a molecular weight of ~30 kb. This genome consist of a 5′ cap structure with a 3′ poly (A) tail, which allows translation of viral proteins including the i) spike (S), ii) membrane (M), iii) envelope (E) and iv) nucleocapsid (N) proteins. The homotrimer of S protein has a molecular weight of 150 kDa, with N-linked glycosylation which forms the spike-like structure on the surface of the virus (3). The M protein has a molecular weight of 25–30 kDa, which has 3 transmembrane domains and gives the virion shape. The E protein is present in small quantities within the virion, has a molecular weight of approximately 8–12 kDa, facilitates assembly and release of the virus, and also has ion channel activity. Another structural protein, the hemagglutinin-esterase, is present in a subset of β-coronaviruses, binds with sialic acids on the surface of glycoproteins, and also has acetyl-esterase activity [7,8].

Table 1
Types of Coronavirus and their host receptors.

Coronaviriniae Genera	Strain	Receptor	host
Alpha-coronavirus	HCoV-229E	Human Aminopeptidase N (CD13)	Bats
	HCoV-NL63	ACE2	Palm Civets, Bats
Beta-coronavirus	HCoV-OC43	9-O-Acetylated sialic acid	Cattle
	HcoV-HKU1	9-O-Acetylated sialic acid	Mice
	SARS-CoV1	ACE2	Palm Civets, Bats
	MERS-CoV	DPP4	Bats, Camels
	SARS-CoV2	ACE2	Bats

3. Viral entry, pathogenicity, and immunity upon coronavirus infections

The virus initially binds to the host cell's receptor via the S protein, and more specifically, the S1 domain/subunit of S protein. Depending on the type of coronavirus, the receptor-binding domains (RBD) within the S1 subunit/region can vary [9]. The S-protein–receptor interaction governs the tissue tropism of the virus. As seen in Table 1 host cells receptors also vary depending on the type of coronavirus [7]. For example, angiotensin-converting enzyme 2 (ACE2) serves as the receptor for the SARS-CoV and HCoV-NL63, whereas dipeptidyl-peptidase 4 serves as the receptor for MERS-CoV. Subsequently, the virus enters the cytosol by acid-dependent proteolytic cleavage of the S protein, primarily by the protease cathepsin, though other proteases can play this role. Finally, fusion of the viral and host cellular membranes occur in the acidified endosome of the host cell, ultimately releasing the viral genome into the cytoplasm. Following replication and assembly, virions are transported to the cell surface through vesicles and released by exocytosis [7]. Upon viral infection with the SARS-CoV, the antigen presenting cells (APC) process the viral antigen and present the processed antigen to the T-cells by MHC class 1 [10,11]. Antigen presentation activates humoral and cellular immunity responses by B and T cells, respectively. The antibody profiles against the SARS-CoV2 virus have a typical pattern of IgM and IgG production. Predominantly, S and N specific antibodies are produced. The SARS-specific IgM antibodies disappear at the end of week 12, whereas the IgG antibodies can last for a long time, suggesting that the IgG antibodies may have a protective role [12,13]. From infection to onset of symptomatic illness generally occurs within 12 days. The clinical symptoms of COVID-19 are heterogeneous and range from mild flu-like symptoms to rapidly developing acute respiratory distress syndrome (ARDS), respiratory failure, sepsis driven cardiac injury and arrhythmias, septic shock, and multiple organ failure, which can eventually cause death [14–17]. The most common symptoms of COVID-19 are fever, cough, headache, fatigue, shortness of breath and leukopenia. Nausea and diarrhea can also occur, but are observed less commonly. Currently, the detection of SARS-CoV-2 viral

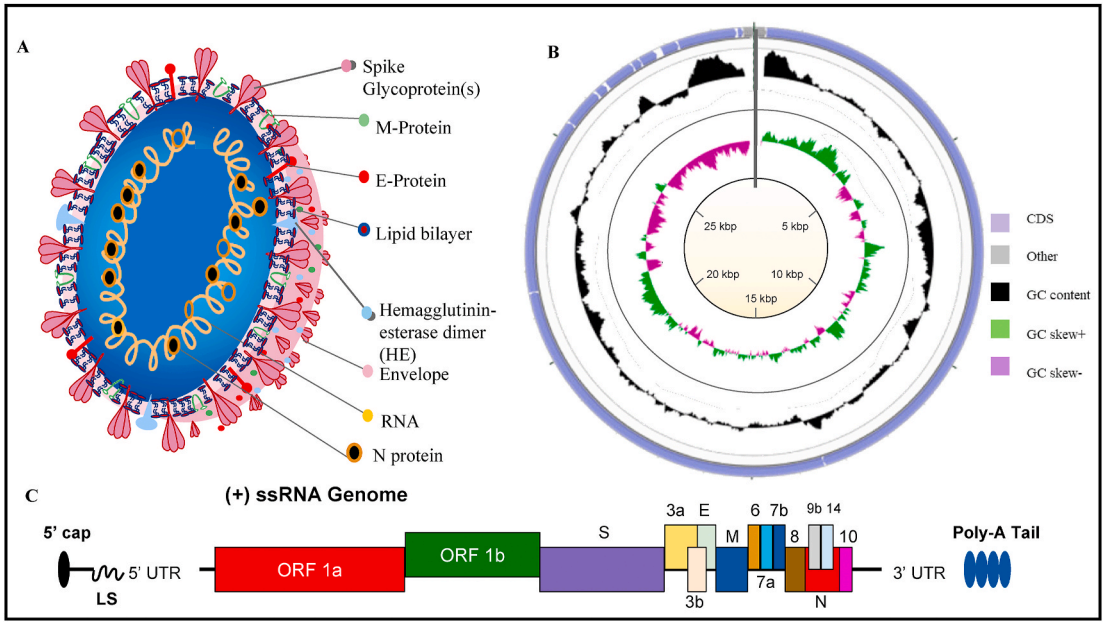

Fig. 1. SARS-CoV-2 virus structure (A) Architecture of the of SARS-CoV-2 genome. Representation of the reference genome of SARS-CoV-2 showing the protein-coding regions and GC content of the genome is sh own (B) Representation of 5′ capped mRNA. The mRNA has a leader sequence (LS), poly-A tail at 3′ end, and 5′ and 3′ UTR. It consists of ORF1a, ORF1b, Spike (S), ORF3a, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, Nucleocapsid (N), and ORF10 (C) [8].

RNA is the primary method for diagnosis of COVID-19. However, measurement of serological titers of IgM and IgG [17] as well as detection of SARS-CoV-2 nucleocapsid protein (NP) antigen by fluorescence immunochromatography also shows high specificity and relatively high sensitivity in the early phase of the infection [18]. Several studies have reported that soon after respiratory viral infection and following injury induced by the virus, bacterial colonization and super-imposed infection occurs at the basement membrane of the respiratory epithelium. In a mouse model, Ami et al. reported that SARS-CoV infection together with low-virulent Pasteurella neurotropic bacterial infection and lipopolysaccharide derived from *Escherichia coli* enhanced the replication of SARS-CoV, causing the exacerbation of respiratory disease by inducing elastases in the lungs, a finding that was associated with a high mortality rate in these animals [19]. In support of this finding, bacterial superimposed infections are commonly observed in COVID-19 patients [20–24]. Furthermore, antibiotic resistant bacterial species have been reported in a subset SARS-CoV and SARS-CoV-2 infected patients [25,26] and may lead to superimposed bacterial pneumonia.

4. Current treatment strategies for COVID-19

Several antiviral treatment strategies are currently being used for the treatment of COVID-19. Remdesivir is an adenosine analog which inserts into the viral RNA during replication, causing premature termination of viral RNA. It has been reported that seriously ill COVID-19 patients who received remdesivir had reduced rate of invasive mechanical ventilation (17 of 30 patients) & extracorporeal membrane oxygenation (3/4)[22]. A randomized clinical trial of 1063 COVID-19 patients who received remdesivir vs. placebo showed a significant reduction in the recovery time with evidence of lower respiratory tract infection [27]. A different antiviral drug, Favipiravir, impairs viral RNA polymerase activity, interfering with viral genome replication. favipiravir has to undergo phosphorylation to convert it to its active form, (Favipiravir-RTP). Treatment with favipiravir was shown to increase SARS-CoA-2 viral clearance and demonstrated improvement in radiographic findings of COVID-19 patients as compared to those treated with lopinavir/ritonavir [28]. Several randomized trials have been initiated to test the efficacy of favipiravir either combined with other drugs or alone in treatment of COVID-19[29]. EIDD-2801 is an orally bioavailable form of the antiviral compound EIDD-1931 (a ribonucleotide analogs that introduces copy errors during virual replication). EIDD-2801 treatment enhanced pulmonary function and significantly reduced the viral titers of SARS- and MERS-CoV–infected in the lungs of mice [30]. The efficacy of EIDD 2801 is being investigated in randomized clinical trials in COVID-19 patients [29]. Lopinavir/ritonavir (LPV-r) interfere with the viral protease that processes large polypeptide chains after protein synthesis into individual functional proteins. A retrospective analysis initially demonstrated that administration ($\leq$10 days from disease onset) of lopinavir/ritonavir shortened the duration of viral shedding in COVID-19 patients [31]. However, a randomized trial of lopinavir/ritonavir did not show efficacy in treatment of SARS-CoV-2 [32].

A secondary treatment strategy aims to reduce the inflammatory response that occurs in the setting of COVID-19 and is the cause of significant morbidity and mortality. Tocilizumab (TCZ) specifically binds to soluble and membrane-bound IL-6 receptors (IL-6R), leading to the reduction of IL-6 mediated pro-inflammatory signaling. TCZ treatment improved clinical status, such as hypoxemia and severity of CT lung imaging findings, in severe COVID-19 patients in a small observational study [29,33]. A retrospective study suggested that TCZ treatment reduced inflammatory markers, such as IL6 and C-reactive protein, in moderate-to-critically ill COVID-19 patients [29]. Further, different retrospective cohort studies have revealed that TCZ treatment significantly reduced the duration of vasopressor support in hypoxic COVID-19 patients compared to patients who did not receive TCZ [29]. However, Roche's phase III clinical trial of TCZ failed to show improvements in recovery or mortality when used alone for treatment of COVID-19 [34]. On the other hand, a non-randomized study evaluating the benefit methylprednisolone (a steroid) followed by TCZ treatment (in case of insufficient improvement) compared to historical controls showed accelerated respiratory recovery, decreased hospital mortality rates, and reduced invasive mechanical ventilation in COVID-19-associated cytokine storm syndrome [35]. Therefore, the role of TCZ at this point of time in treatment of COVID-19 is unclear. Finally, dexamethasone, a different steroid, has also been used to reduce the inflammatory response in COVID-19 pateints. A recent study suggests that COVID-19 patients who have received dexamethasone had a lower incidence of death as compared to usual care [36]. Finally treatment of bacterial superimposed infections in COVID-19 patients is important. Hence, the antibiotic, azithromycin is also often administered in the treatment of COVID-19. This antibiotic directly interferes with bacterial protein synthases [3,37–40], [41].

5. CQ and HCQ

Much enthusiasm initially surrounded CQ and HCQ with or without azithromycin for treatment of COVID-19, given results of *in vitro* studies. CQ and HCQ increase the pH of cellular endosomes/lysosomes (leading to alkalization of endosomes), which subsequently impairs fusion, and eventually blocks the release of viral RNA into the cytosol [42]. Impairment of endosome/lysosome fusion, however, also affects the normal function of host cells, including authophagy. Autophagy is an intracellular recycling pathway that maintains the integrity of intracellular organelles via endosomes and lysosomes. This process removes microbes, damaged organelles and proteins, and oxidative products, maintaining cellular homeostasis [43–45]. As mentioned above, following viral/bacterial infections, APC such as macrophages and dendritic cells process viral/bacterial antigens and subsequently APC present the processed antigen to T cells by MHC molecules. This antigen processing and presentation by MHC molecules to T-cells is in part, an autophagy dependent process [46,47]. Autophagy links both the innate and adaptive immune systems, which includes thymic selection, antigen presentation, maintenance of lymphocyte homeostasis and survival, and regulation of cytokine production during bacterial/viral infections [48]. Thus, activation of autophagy maintains viability of organs and immune cells. CQ and HCQ are known to inhibit the autophagy process by blocking autophagosome and lysosome fusion, ultimately leading to suppression of immune cell function (Fig. 2). By interfering with the autophagy process, CQ and HCQ treatment may also lead to both immune and non-immune cell death. The critical role of autophagy in host immune response was demonstrated in autophagy deficient mouse models, which demonstrated impairment of T and B cell activation, proliferation, differentiation, and function with aging [49–51]. The host's innate immune system identifies coronavirus infection through Toll-like receptors (TLRs) [52,53]. It has been reported that compared to wild type mice, TLR3 $^{-/-}$, TLR4$^{-/-}$, and TRAM$^{-/-}$ (TRIF-related adaptor molecule) deficient mice were more susceptible to SARS-CoV infection and showed increased weight loss transiently. However, mice deficient in the TRIF (TIR {Toll/interleukin-1 receptor}Domain-containing Adaptor-inducing Interferon-B) adaptor protein (the adaptor protein for TLR3 and TLR4) were highly susceptible to SARS-CoV infection, as manifested by a significant weight loss, reduced lung function, and increased lung pathology and mortality [54]. Upon activation, these pattern recognition sensors initiate a signaling cascade that leads to the expression of type I IFN and other inflammatory cytokines that limits viral replication through a variety of mechanisms. TLRs localize to endosomal compartments, where they recognize microbial nucleic acid [55]. As acidification of the endosome is necessary for TLR mediated antiviral function, it is possible that HCQ amd CQ may also block TLR mediated antiviral/antibacterial pathways (Fig. 2).

Other potential mechanisms for the lack of benefit (and even presence of harm) for HCQ and CQ in the setting of COVID-19 may be due to their effects on ACE-2 and pulmonary surfactants. Though ACE2 serves

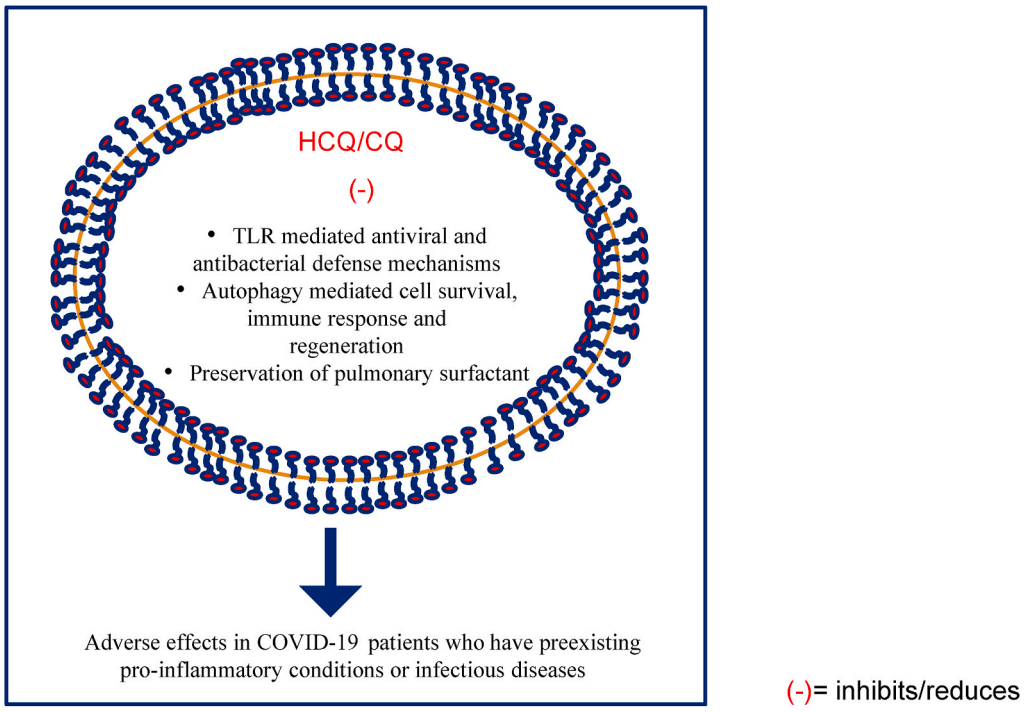

Fig. 2. CQ/HCQ can inhibit function of host cells. CQ/HCQ potentially inhibit autophagy mediated cell survival, immune response, cellular regeneration, and TLR mediated antiviral and antibacterial mechanisms, and alter pulmonary surfactants by changing the pH of endosomes. This can potentially lead to adverse effects in COVID-19 patients who have preexisting inflammatory or infectious conditions.

as a receptor for SARS-CoV-2, it has a biologically important role, converting angiotensin II (vasoconstrictor peptide) to angiotensin1-7 (vasodilator). Deficiency of ACE2 expression/activity increases angiotensin II levels, which further triggers interstitial fibrosis, endothelial dysfunction, inflammation, obesity-associated hypertension, and coagulation. In a lung injury model, spike protein of SARS-CoV was shown to downregulate ACE2 levels, which lead to further deterioration of lung function, an effect that was ameliorated by angiotensin–receptor blocker medications [56]. HCQ and CQ have been reported to reduce the glycosylation of ACE2 in host cells, which is essential for its activity [57–59]. In addition, by interfering with endocytosis, HCQ and CQ can inhibit pulmonary surfactant production. Pulmonary surfactant are macromolecules secreted by exocytosis of lamellar bodies and recycled via endocytosis by type II alveolar epithelial cells. These macromolecules are critical in controlling the alveolar surface tesnsion to maintain optimal gas exchange and prevent alveolar collapse at end-expriation [60,61].

For the initial immune response, phagocytosis must occur in order to kill viruses and bacteria. This requires an acidic environment. CQ and HCQ inhibit phagocytosis by decreasing acidification. Impaired phagocytosis can lead to accumulation of bacterial pathogens, causing superimposed secondary bacterial infection, and worsening the course of disease. In fact, COVID-19 patients have been reported to suffer from super-imposed bacterial infections, including *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, Acinetobacter, and *Klebsiealla pneumoniae* [20,26]. At the time of initial viral infection, macrophages and neutrophils are activated and help eliminate pathogens. During phagocytosis, reactive oxygen species (ROS) are released from these cells, resulting in oxidation of various lipids and proteins in the cell and neighboring tissues or organs [62–64]. If the process of autophagy fails to clear these oxidized proteins and lipids, they can interefere with many aspects of cellular function. For example, i) oxidized HDL reduces its cholesterol efflux capacity [65,66], ii) oxidation of thiol groups of cysteine residues in the ryanodine receptor 2 increases its activity in myocytes and can be proarrhythmic [67], iii) nitrosylation of p85 protein, the regulatory subunit of PI3-kinase has been shown to inhibit phagocytosis in macrophages [68], iv) oxidatively modified antioxidant enzymes inhibit their own enzymatic activity [69], further amplifying a prooxidant state and oxidative stress [70,71]. It has been well documented that the elderly and patients with cardiovascular disease have an increased state of oxidative stress and inflammation [62,72]. COVID-19 patients who are elderly or have cardiovascular disease have a surplus of prooxidants [73], which may predispose to cytokine storm by activating nuclear transcription factor-kappa B. The critical role of autophagy in the inflammatory response is also demonsrated by studies that have shown that autophagy related gene 5 (ATG5) expression levels are decreased with severity of sepsis, and ATG5 polymorphisms (rs506027 T > C and rs510432 G > A) are associated with sepsis progression and mortality. Deficiency of autophagy-related genes aggravate inflammatory diseases, including in the setting of pulmonary, inflammatory bowel, cardiovascular, and neurodegenerative diseases [74–78]. Therefore, CQ and HCQ *in-vivo* might actually promote inflammation and progression of sepsis by interfering with autophagy and clearance of oxidized molecules.

It is also important to note that for treatment of COVID-19, HCQ and CQ cytotoxic and viral replication inhibition assays were tested with only one type of cell line (Vero, kidney cell line) [42]. Further, though an *ex vivo* study demonstared that HCQ and CQ reduce viral replication, a recent study suggested that HCQ treatment showed no benefit in hamsters and macaques infected with SARS-CoV-2 [79]. In fact, studies by Stuart et al. and Falzarano et al. revealed that CQ inhibited replication of the Ebola virus *ex vivo*, but failed to protect against infection *in vivo* in guinea pigs, mice, and hamsters [80,81]. Vigerus and colleagues showed that *ex vivo*, CQ is effective against influenza A virus, but not *in vivo* [82].

Though initial clinical studies suggested a modest benefit for HCQ in treatment of COVID-19, rigorous followup randomized clinical trials did not confirm these results and showed no clinical efficacy. Gautret *et al.* showed HCQ treatment in patients with COVID-19 was associated with a significant decrease in viral load and its effects were reinforced by azithromycin. This study, however, had a small sample size (control

patients n = 16, HCQ = 20, azithromycin = 4) and did not evaluate clinical outcomes [83]. In a recent randomized controlled trial, HCQ alone or combination of HCQ with azithromycin (Control patients N = 227, HCQ N = 221, HCQ and azithromycin N = 221) showed no clinical benefit for treatment of COVID-19[4]. Moreover, patients who received HCQ plus azithromycin (39.3%) or HCQ alone (33.7%) had more adverse events than those who received azithromycin alone (18.0%) or routine care (22.6%). Further, low lymphocytes levels and increased markers of liver injury, such as alanine aminotransferase and aspartate aminotransferase, were observed in patients who received CQ or HCQ plus azithromycin than in the control group. In addition, QTc prolongation was noted in both the HCQ plus azithromycin (39.3%) and HCQ groups [4].

HCQ, and at times, CQ, are used for treatment of rheumatoid arthritis and malaria, and their use is well supported by pre-clinical studies [84,85]. However, unlike COVID-19, rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial inflammation and joint destruction. HCQ is used to suppress immune cell function [86]. Underlying lung and other organ dysfunction/failure is not commonly associated with RA, as it is in COVID-19, in whom notable heart and lung injury as well as secondary lymphoid organs damage have been reported [87]. Therefore, results observed in treatment of RA are not directly applicable COVID-19. Of note, HCQ has been reported to increase risk of heart failure in patients with rheumatoid arthritis [88]. A recent multicenter, retrospective study of HCQ for treatment of rheumatoid arthritris demonstrate that although HCQ had no excess risk of severe adverse events in patients acutely, long term treatment was associated with excess of cardiovascular mortality [89]. It has been also reported that administration of CQ and its analogs long-term cause adverse effects on vision, including keratopathy and retinopathy, in patient with rheumatoid arthritis [91,92]. Finally, CQ treatment is associated with ototoxicity, including sensorineural hearing loss, tinnitus, and cochleovestibular manifestations [93].

Both HCQ and CQ are also used to treat malaria. In this setting, HCQ and CQ alkalinize the food vacuoles in the plasmodium parasite, which eventually blocks the digestive mechanisms of the parasite, leading to its death. In addition, HCQ and CQ inhibit the bio-crystallization of the toxic heme molecule (from the hemoglobin derived from the host blood) to nontoxic hemozoin within the parasite, and high accumulation of heme contributes to parasitic death [90]. These mechanisms of action significantly differ from those in COVID-19 patients, where HCQ and CQ are taken up primarily by host cells. Further, malaria patients rarely present with cytokine storm and severe multi-organ dysfunction, as it is observed in COVID-19.

Finally, HCQ treatment has been studied in the setting of HIV infection. HCQ treatment resulted in reduced CD4 cell counts and increased HIV viral load in patients with chronic HIV infection, who were not receiving antiretroviral therapy, compared to placebo [94].

6. Potential strategies to treat COVID-19 effectively

Currently, there is no specific effective therapy for COVID-19. Based on existing evidence, the therapeutic approach should include a combination of 1) eradication of the virus by inducing an innate immune response, 2) inhibition of viral replication, 3) destruction of bacteria by antibiotic regiments, and 4) clearance of inflammation and inflammatory precursors without affecting the host' cell cell function. In addition to remdesivir (which inhibits viral replication), adjunctive or preventive strategies (Fig. 3) may also include supplementation with agents that are known to help immune function, including vitamin A, C, and E, Zinc, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). These supplements can enhance innate immune function and reduce inflammation [22,27,95]. Supplementation with vitamin C induces chemotaxis and phagocytosis in macrophages and neutrophils [96]. Several studies have documented that vitamin C enhances proliferation of B- and T-cells, by regulating gene expression involved in proliferation and differentiation [96–98]. Further, clinical studies have suggested that vitamin C ameliorates flu-like symptoms and possesses viricidal

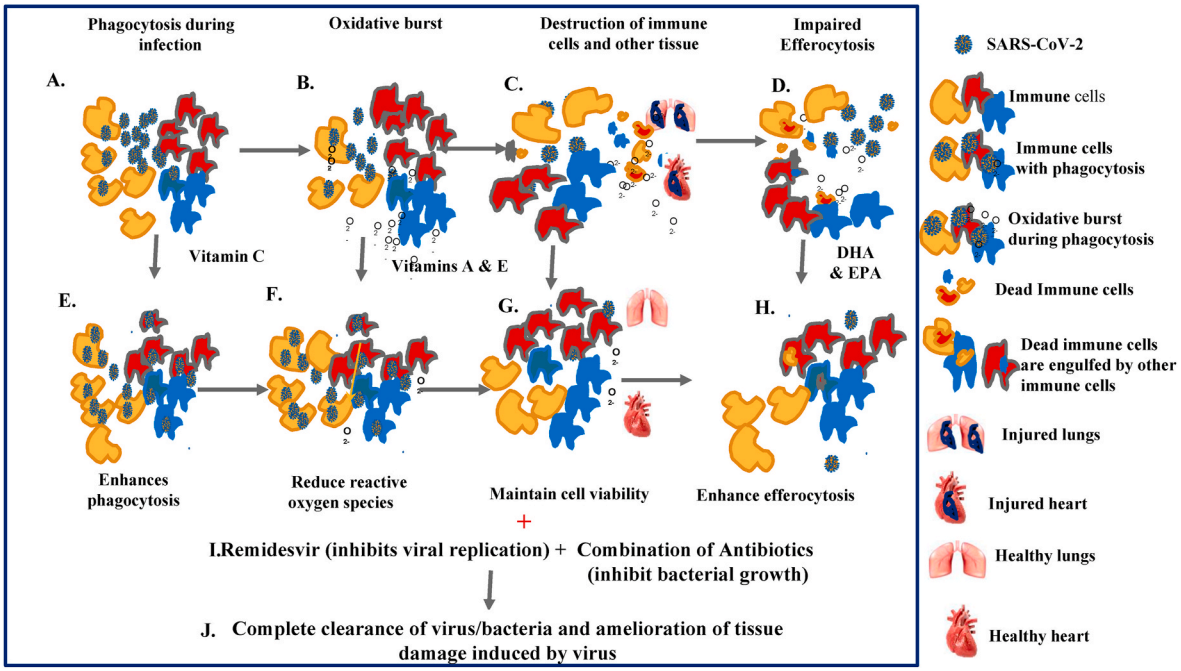

Fig. 3. Schematic presentation of a potential strategy to treat COVID-19. (A) With viral infection, host immune cells initially engulf the SAR-CoV-2 virus via phagocytosis. (B) Phagocytosis is intended to kill pathogens, but this processes causes the release of ROS. (C) ROS damage host immune cells and lead to tissue injury and death (or) less viable cells. (D) Injured and dead cells are engulfed by immune cells through the efferocytosis. (E)Vitamin C and Zinc enhances phagocytosis, allowing for subsequent destruction of a greater number of pathogens. (F–G) Released ROS can be potentially quenched by vitamin A and E, resulting in decreased cell death and reduced collateral cardiac and lung injury. (H) DHA and EPA enhance efferocytosis. (I) Remdesvir inhibits viral replication and combined antibiotic inhibit the bacterial growth. (J) The combination of the above strategies can lead to viral clearnce and amelioration of tissue damage by the virus.

properties compared to a control group [99]. Furthermore, Zinc supplementation induces T cell proliferation, increases phagocytosis in macrophages and neutrophils, and enhances natural killer cell activity [100]. Several clinical studies have shown that supplementation with zinc can reduce the risk of pneumonia and the incidence of respiratory tract infection in the elderly and in children [101,102]. [103,104]. Though vitamin C and zinc enhance phagocytosis, they are often insufficient to counteract the effect of ROS, which are formed during the phagocytic process [64,70]. Hence, administration of other antioxidants (Vitamins A and E) may be important to alleviate the oxidative burst that is formed during phagocytic processes, thereby preventing organ injury and cell death [105]. [106–108]. Further effective clearance of dead cells, namely "efferocytosis," is the primordial path for organ development/regeneration, maintaining cellular homeostasis, and resolving inflammatory insults [109]. Inevitably, SARS-CoV2 causes increased cell death, including immune cells. DHA, an omega-3 fatty acid, can increase the efferocytosis processes by forming resolvin D1 (RvD1) [110,111]. Finally, if secondary infections are identified in clinical cultures, antimicrobial susceptibility testing has to be evaluated in clinical samples and specific antibiotic/combination of antibiotics (susceptibility of bacteria to specific antibiotic/combination of antibiotics) should be given. Indeed some infections described in patients with COVID-19 are instigated by drug-resistant organisms, such as *Klebsiealla pneumoniae*, *Pseudomonas aeruginosa*, extended spectrum beta-lactamase, multidrug resistant *E. coli*, *Mycoplasma pneumoniae*, and Acinetobacter [20]. Emerging studies suggest that drug resistant microbes such as *Klebsiealla pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* are common nosocomial infections in intensive care units [112,113].

7. Conclusions

This review provides insight as to how HCQ and CQ treatment might interfere with the immune system and redox status of COVID-19 patients, especially those who are elderly or suffer from co-morbidities. It also emphasizes the importance of recognizing and controlling superimposed bacterial infections. Hence, developed therapies against SARS-CoV2 should employ strategies that protect immune cell functions from oxidative stress and superimposed bacterial infections.

Declaration of competing interest

The author declares that there are no competing interests associated with this manuscript.

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