Pharmacology of CQ/HCQ and COVID-19

Subjects: Physiology

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Chloroquine (CQ) and hydroxychloroquine (HCQ) have been proposed as treatments for COVID-19. These drugs have been studied for many decades, primarily in the context of their use as antimalarials, where they induce oxidative stress-killing of the malarial parasite. Less appreciated, however, is evidence showing that CQ/HCQ causes systemic oxidative stress. In vitro and observational data suggest that CQ/HCQ can be repurposed as potential antiviral medications.

Keywords: chloroquine ; hydroxychloroquine ; proton fluxes ; COVID-19 ; oxidative stress ; cytokine storm ; SARS-CoV-2 ; macrophage ; immune response ; reactive oxygen species

1. Introduction

At the writing of this work (mid-2020), there was no Food and Drug Administration (FDA) approved drugs for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA virus infection, which causes the coronavirus disease 2019 (COVID-19). Both chloroquine (CQ) and hydroxychloroquine (HCQ) are 4-aminoquinoline drugs with similar structures, as shown in Figure 1. CQ and HCQ have been proposed as potential COVID-19 medical countermeasures [1][2][3][4][5][6]. The FDA currently approves CQ/HCQ for use as antimalarials, the treatment of rheumatoid arthritis, and systemic lupus erythematosus (SLE) where they act by immunomodulatory mechanisms [7]. HCQ reduces disease activity in SLE patients but has no significant effect on pro-inflammatory cytokines [8]. A recent literature review highlights many immunomodulatory mechanisms influenced by CQ/HCQ and emphasizes our lack of current knowledge concerning their potential effects on immune responses in COVID-19 patients [9]. CQ/HCQ use may lead to unknown alterations in immune responses in COVID-19 patients, including diminished innate immune responses as well as potential modifications of the B and T cell responses to the COVID-19 virus [9].

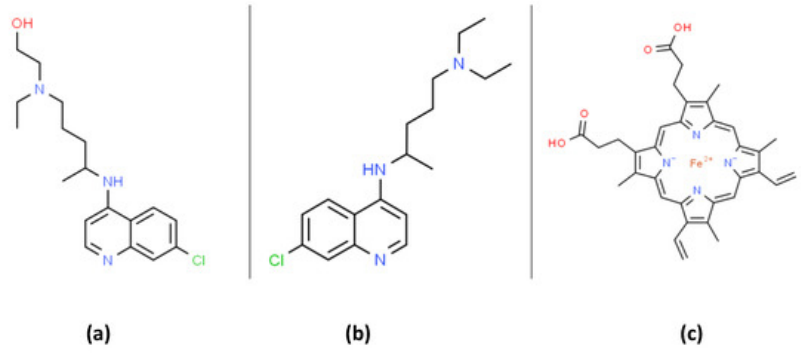

Figure 1. The organic structures of (a) hydroxychloroquine (HCQ); (b) chloroquine and; (c) heme. Chloroquine can form a membrane bound complex with heme that can promote lipid peroxidation.

Both in vitro and in vivo animal experiments have demonstrated the anti-coronavirus activity of CQ /HCQ [10][11][12]. Moreover, CQ/HCQ are broadly available, cost-effective, and therefore attractive potential therapies for viral pandemics without an effective vaccine. A review by Cortegiani et al. on the efficacy and safety of CQ for treating COVID-19 concluded that available pre-clinical evidence was sufficiently robust to justify initiating high-quality clinical trials [4]. Based on available data, the FDA initially published an emergency use authorization of CQ/HCQ (on 28 March 2020) for COVID-19 treatment. The FDA subsequently revoked this emergency use authorization (on 15 June 2020) due to new clinical data suggesting CQ/HCQ were ineffective and raising concerns regarding "serious cardiac adverse events and other potential serious side effects" [13]. Nevertheless, the FDA states "additional clinical trials continue to evaluate the potential benefit of these drugs in treating or preventing COVID-19. "The molecular basis for the potential antiviral activity of CQ/HCQ are not fully understood, and multiple mechanisms have been proposed [9]. This review will explore the hypothesis that alterations in proton fluxes and redox physiology induced by CQ/HCQ are relevant to their potential antiviral activity and side-effects. The production of reactive oxygen species (ROS) and cellular/subcellular proton fluxes

are interdependent factors modulating many antimicrobial immune responses [14]. We will also highlight concerns that CQ/HCQ-induced oxidative stress could be problematic in the treatment of critically ill COVID-19 patients. The "cytokine storm" occurring in some severe forms of viral infection (e.g., influenza A viruses) is associated with increased oxidative stress as well as increased morbidity and mortality [15]. Accumulating evidence suggests that this cytokine storm is a significant cause of ARDS and multiorgan failure in COVID-19 patients [16]. Considerable evidence suggests that parasite-specific oxidative stress induced by CQ/HCQ treatment accounts for the antimalarial activity of these drugs [17]. Less appreciated, however, is evidence (see below) showing that CQ/HCQ, by themselves, are pro-oxidants that can increase oxidative stress parameters [18][19][20][21]. Viral infections are also generally accompanied by oxidative stress with potential pathophysiological consequences [22]. As mentioned above, compelling evidence supports the view that patients with COVID-19 are at increased risk of developing ARDS and subsequent death from respiratory failure [23]. Decades of research have demonstrated the central role of oxidative stress in ARDS pathophysiology [24][25][26][27]. Understanding the potential role of oxidative stress in COVID-19 is critical, since potential anti-COVID-19 drug candidates that increase oxidative stress have the potential to exacerbate ARDS pathophysiology.

2. CQ/HCQ Pharmacology and Alterations in Subcellular Organelles Proton Fluxes

Before reviewing the role of CQ/HCQ in redox physiology and SARS-CoV-2 infection, we will briefly summarize the basics of CQ/HCQ pharmacology (see Figure 2) with an emphasis on CQ/HCQ-induced alterations in subcellular organelle proton fluxes. CQ/HCQ-induced changes in proton fluxes will have a direct effect on the type of ROS present, and thereby influence their potential effects on redox-sensitive signal transduction pathways and potential molecular and cellular damage. Among the key redox-sensitive pathways are those controlled by transcription factor nuclear factor erythroid 2 p45-related factor 2 (NRF2), hypoxia-inducible transcription factors (HIFs), nuclear factor-κB (NF-κB) transcription factors, and activator protein-1 (AP-1) transcription factors (see below as well). Excellent, comprehensive reviews of CQ/HCQ pharmacokinetics and their side-effects are available [9][28][29].

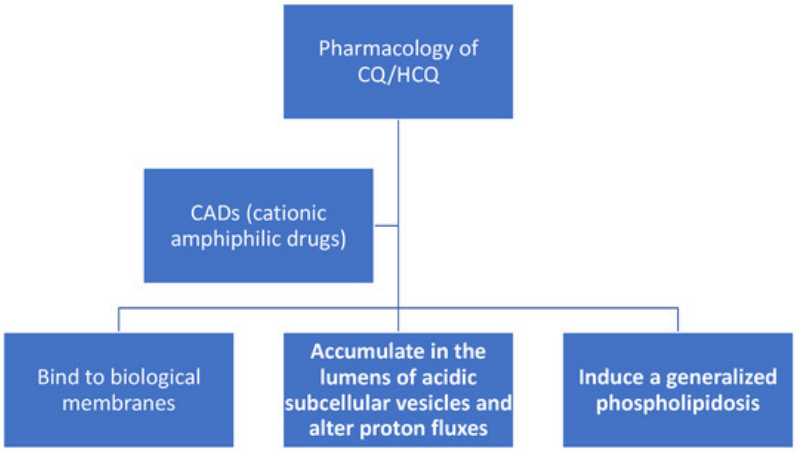

Figure 2. Chloroquine (CQ) and hydroxychloroquine (HCQ) are cationic amphiphilic drugs (CADs) that share a set of common pharmacological properties: (a) they can partition into biomembranes; (b) accumulate in the lumens of acidic subcellular organelles, and; (c) induce a generalized phospholipidosis, which is the lysosomal accumulation of phospholipids.

3. CQ/HCQ Bind to Biological Membranes and Alters their Structure/Function

After many decades of research, some of the least controversial characteristics of CQ/HCQ are the ability of these weakly basic and lipophilic compounds: (1) to bind biological membranes; (2) to accumulate in the lumens of acidic subcellular vesicles and alter proton fluxes; (3) to induce generalized cellular phospholipidosis. These effects are relevant to the production of ROS, proton fluxes and the immunomodulatory and potential antiviral activities of CQ/HCQ.

CQ and HCQ both belong to a class of compounds (see Figure 1 and Figure 2) termed cationic amphiphilic drugs or CADs [30]. In plasma (pH 7.4) and cellular cytoplasm (pH 7.2), the divalent forms of CQ/HCQ are the dominant species. At pH 7.4 CQ, CQH^+ (monovalent), and CQH_2^{2+} (divalent) are present at 0.026, 16.74, and 83.23%, respectively (reaction 1). Unambiguous in vitro experiments show that CQH_2^{2+} binds to the phospholipid bilayers of multilamellar liposomes with a robust partition coefficient [31]. Phosphatidylserine (PS) liposomes, with a negative charge, binds CQH_2^{2+} with a

particularly high partition coefficient [31]. PS plays a central role in apoptosis. Proton NMR studies have confirmed the binding of CQH_2^{2+} to phosphatidylcholine liposomes [32]. As expected, resonance signals from protons on the hydrophobic ring carbons of CADs are affected by association with liposomes [32].

The binding of CADs to lipid bilayers is stabilized by nonspecific hydrophobic and ionic interactions [31]. Lipid bilayers are present in all cells and many subcellular organelles. It follows that CADs can accumulate, to some extent, in the cells and membranous organelles of most tissues. CAD accumulation can potentially affect many functions of all biological membranes by altering their structure and functions, e.g., membrane-bound lysosomal phosphatases and hydrolases [33].

4. CQ/HCQ Accumulate in the Lumens of Acidic Subcellular Vesicles and Alter Proton Fluxes

It is also well established that CADs accumulate in the lumens of acidic subcellular vesicles. The neutral forms of CADs can freely diffuse through the hydrophobic domain of lipid bilayers. For acidified vesicles, such as endosomes, lysosomes, M2 phagosomes, and pulmonary lysosomal lamellar bodies, the neutral forms of CADs will become charged inside the lumen and will no longer be able to diffuse out [28]. Acidified vesicles utilize a vacuolar proton-pumping ATPase (V-ATPase) to maintain a low pH by pumping protons across the vesicular membrane into the lumen [34]. For lysosomes attempting to maintain a low pH (pH 4.8) in the presence of CQ/HCQ, there will be a gradual luminal accumulation of CQ/HCQ, eventually leveling off at levels as high as 20 mM [28][35]. This process is termed "lysosomal trapping," and trapped CADs are denoted as "lysosomotropic" [35][36][37]. CQ shows a wide variation in lysosomal trapping between organs with lungs > kidney = brain = liver > diaphragm = heart = skeletal muscles > adipose tissue [38]. The high CQ accumulation in the lungs is relevant to respiratory distress disorders [39]. Lamellar bodies are a type of acidified lysosome found in type II alveolar cells (T2ACs), and keratinocytes and these organelles are known to accumulate weak bases such as CQ/HCQ [40][41]. The lamellar bodies found in T2ACs secrete surfactant, which is essential for pulmonary alveoli gas exchange [42]. T2ACs are particularly relevant to COVID-19, since these cells express angiotensin-converting enzyme-2 (ACE-2), which SARS-CoV-2 utilizes as a receptor to enter the lungs. T2AC cells are preferentially infected by SARS-CoV-2, potentially contributing to a reduced secretion or function of surfactant with a resulting loss of pulmonary compliance [43]. Reduced pulmonary compliance is a typical characteristic of ARDS, but exogenous surfactant treatment has not proven to be therapeutically effective [44][45]. Nevertheless, there is a pharmaceutical interest in testing surfactant therapy in COVID-19 patients. CQ interferes with the processing of surfactant protein C, which is necessary for a fully functional surfactant [46]. Moreover, ROS can inactivate surfactant by structural and functional modifications to surfactant proteins SP-B and SP-C [47]. Initial in vitro data suggested that the pH of lysosomes would increase as a result of CAD trapping, but subsequent, more detailed studies do not support this view [30][35][48][49]. Data in an animal model show, for example, that CQ (40 mg/kg body weight) will transiently increase hepatocyte lysosomal pH from 4.8 to 6.8 for about 2 h, followed by a return to pH 4.8 lasting for at least 10 h [49]. Maintaining an acidic lysosomal pH in the face of CQ accumulation necessitates an increased ATP consumption by V-ATPase and an increased proton flux into the lysosomal lumen.

5. Endosomal-Lysosomal Proton Fluxes, CQ/HCQ and SARS-CoV-2

A third well-studied effect caused by CADS (see Figure 2) is a generalized lysosomal accumulation of phospholipids termed phospholipidosis. Phospholipidosis is a lysosomal storage disorder characterized by an abnormal accumulation of phospholipids in the form of lamellar bodies [30]. Drugs causing phospholipidosis are recognized as being potentially toxic by the pharmaceutical industry [50][51]. The underlying mechanism(s) for phospholipidosis remains a matter of some controversy. Most likely, CAD inhibition of lysosomal lipid degradation enzymes is involved [30]. Studies in an animal model indicate that CADs can induce pulmonary lesions characterized by large foamy macrophages in the alveolar spaces [51]. The foamy alveolar macrophages show a typical CAD-induced lysosomal phospholipidosis with the potential to interfere with the phagocytosis and catabolism of pulmonary surfactant [51]. The potential pathophysiological consequences of phospholipidosis remain an area of active interest. The view that lysosomes are relatively inert organelles with a narrow degradative function is rapidly changing [36]. We now appreciate that lysosomes are dynamic organelles playing a central role in a wide variety of signaling pathways affecting immune responses, viral infectivity, the inactivation of microbes, and inflammation [35][52][53]. Alterations in luminal proton fluxes can be induced by both CQ/HCQ and ROS (see below). Proton fluxes can, in turn, influence both the levels and types of ROS [54]. Lysosomes can fuse with endosomes, thereby delivering the endocytosed cargo to lysosomes [55]. Pioneering work by Burkard et al. shows that coronavirus entry into cells can occur via this endolysosomal (or endocytic) pathway [56]. The endolysosomal pathway is under intense scrutiny due to its role as a target for COVID-19 therapy [57]. The spike glycoprotein (S) of SARS-CoV-2 is proteolytically cleaved by cellular serine protease TMPRSS2 into two subunits, S1 and S2 [58]. The S1 viral protein binds to the host cell

angiotensin-converting enzyme 2 (ACE2) plasma membrane protein. The SARS-CoV-2 interaction with ACE2 initiates the formation of clathrin-coated pits, which serve as SARS-CoV-2 entry receptors. The virus is then brought into the cell's cytoplasm via endocytosis with the formation of early endosomes. The early endosomes with SARS-CoV-2 subsequently form late endosomes that fuse with lysosomes. The S2 protein subunit promotes the fusion of the viral membrane with cellular membranes [57][58][59]. Encouragingly, sera from recovered SARS patients can block SARS-CoV-2 host cell entry in a cell culture model [58]. The notion that CQ/HCQ could alkalinize endosomal-lysosomal pH and thereby inhibit the replication of viruses requiring an acidic pH has been an attractive hypothesis [60]. This potential mechanism is often proposed as a rationale for the CQ/HCQ treatment of COVID-19 [61]. Nevertheless, the references cited above cast doubt on this hypothesis, since CQ/HCQ endosomal-lysosomal alkalinization appears to be only transient, lasting only a few hours, but long enough to confound short-term in vitro experiments.As mentioned above, CQ/HCQ are likely to increase cellular ATP consumption and increase proton flux across the lysosomal membrane. Under conditions where ATP production is decreased (e.g., mitochondrial uncoupling or hypoxia) sufficiently to inhibit V-ATPase activity, it might be possible for CQ/HCQ to induce some degree of endosomal-lysosomal alkalinization [62]. Low V-ATPase protein expression could also cause CQ/HCQ alkalinization. This avenue of research is not well studied. Intracellular ATP levels are also a key determinant governing the mode of cell death: lack of ATP favors necrosis over apoptosis [63]. Although beyond the scope of this review, it should be noted that many viruses have evolved molecular mechanisms to modulate modes of cell death to their advantage [64]. Blocking apoptosis and the subsequent killing of virally infected cells is one such mechanism [64].

6. Conclusions

The potential role of ROS in COVID-19 remains to be fully elucidated. In the case of influenza A viruses, inhibition of ROS production can reduce inflammation, as measured by a decrease in virally induced cytokine production [15]. The measurement of oxidative stress parameters in CQ/HCQ COVID-19 trials would be of clinical value. Quantification of plasma F2-isoprostanes, specifically 8-epi-prostaglandin F2α, is a clinically useful and sensitive assay for assessing in vivo lipid peroxidation and oxidative stress [65]. Similarly, the plasma ratio of α-tocopherol quinine to α-tocopherol is an excellent indicator of antioxidant status [65]. Marcello et al. recently found that two oxysterols, 7-ketocholesterol and 7-beta-hydroxycholesterol, were increased in the serum COVID-19 patients, particularly those with severe signs. These two oxysterols are also useful biomarkers for in vivo lipid peroxidation [66].

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