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Janus sword actions of chloroquine and hydroxychloroquine against COVID-19

Xuesong Chen*, Jonathan D. Geiger

Department of Biomedical Sciences, University of North Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota, United States of America

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ABSTRACT

Chloroquine (CQ) and its analogue hydroxychloroquine (HCQ) have been thrust into our everyday vernacular because some believe, based on very limited basic and clinical data, that they might be helpful in preventing and/or lessening the severity of the pandemic coronavirus disease 2019 (COVID-19). However, lacking is a temperance in enthusiasm for their possible use as well as sufficient perspective on their effects and side-effects. CQ and HCQ have well-known properties of being diprotic weak bases that preferentially accumulate in acidic organelles (endolysosomes and Golgi apparatus) and neutralize luminal pH of acidic organelles. These primary actions of CQ and HCQ are responsible for their anti-malarial effects; malaria parasites rely on acidic digestive vacuoles for survival. Similarly, de-acidification of endolysosomes and Golgi by CQ and HCQ may block severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) integration into host cells because SARS-CoV-2 may require an acidic environment for its entry and for its ability to bud and infect bystander cells. Further, de-acidification of endolysosomes and Golgi may underly the immunosuppressive effects of these two drugs. However, modern cell biology studies have shown clearly that de-acidification results in profound changes in the structure, function and cellular positioning of endolysosomes and Golgi, in signalling between these organelles and other subcellular organelles, and in fundamental cellular functions. Thus, studying the possible therapeutic effects of CQ and HCQ against COVID-19 must occur concurrent with studies of the extent to which these drugs affect organellar and cell biology. When comprehensively examined, a better understanding of the Janus sword actions of these and other drugs might yield better decisions and better outcomes.

1. Introduction

Chloroquine (CQ) and its analogue hydroxychloroquine (HCQ) have long been used for prophylactic treatment against and treatment of malaria; a condition caused by infection of red blood cells with the parasite plasmodium [1,2]. CQ and HCQ are very similar drugs; HCQ has an extra hydroxyl group at the end of the side chain, and both have similar profiles for drug absorption, distribution, metabolism, and excretion. While HCQ is as active as is CQ against Plasmodium falciparum malaria, it is much less active against CQ-resistant malaria [3]. Furthermore, the wide-spread emergence of CQ-resistant strains of malaria parasites has diminished the effectiveness and use of these drugs in malaria management [4,5].

Beside their anti-malaria effects, CQ and HCQ both exert immunomodulatory and immunosuppressive effects; they are useful in the management of rheumatic diseases, lupus erythematosus, and dermatological disorders [6]. Limited preliminary findings have suggested that CQ and HCQ might exhibit antiviral effects against the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [7,8]; the viral cause of the global pandemic coronavirus disease 2019 (COVID-19) [9–11]. The USA Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) for the use of CQ and HCQ in COVID-19 and there are now over one dozen clinical trials and one state-wide clinical trial (South Dakota) on-going to determine their therapeutic effectiveness in patients living with COVID-19. However, and contrary to public pronouncements, these are not safe drugs; CQ more than HCQ have associated with them side-effects and toxicity profiles including cardiotoxicity, ocular toxicity, and neuromyotoxicity [12–15]. When these drugs were being approved for marketing, cell biology was in its infancy and little was known about de-acidification-induced organellar changes. To enhance the discussion of possible use of CQ and HCQ against COVID-19, it is important for the public, for researchers and for clinicians to appreciate better the effects of CQ and HCQ from a modern cell biology perspective.

* Corresponding author.
E-mail address: xuesong.chen@und.edu (X. Chen).

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2. De-acidifying effects of CQ and HCQ on acidic organelles

2.1. CQ and HCQ de-acidify digestive acidic vacuoles of malaria parasites

CQ (pKa₁ = 8.1, pKa₂ = 10.2) and HCQ (pKa₁ = 8.3, pKa₂ = 9.7) are diprotic weak base drugs that are present in protonated or unprotonated forms. Unprotonated forms of CQ and HCQ can diffuse freely across membranes into endolysosomes and Golgi; once protonated, CQ and HCQ are trapped within these acidic organelles [16]. The driving force for intra-vesicular accumulation of CQ and HCQ is proportional to the square of the hydrogen ion gradient; the accumulation is much larger than that of a monoprotic weak base like ammonia chloride, which is proportional to the hydrogen ion gradient [17,18]. Therefore, CQ and HCQ are preferentially concentrated in and raises the pH of acidic digestive vacuoles (pH of 5.2) of plasmodium parasites that cause malaria [19–21]; the anti-parasitic actions are due to preventing the polymerization of heme into hemozoin [22].

2.2. CQ and HCQ de-acidify acidic organelles in mammalian cells

Modern cell biology is populated by extensive findings about the structure and function of membrane bound organelles that contain a plethora of channels, receptors and transporters regulating organellar function and the biology of the cell. The selective permeability of the organelar membranes creates a unique luminal microenvironment; one that is distinct from the environment of the surrounding cytosol. For acidic organelles, a key requirement for optimal function is maintenance of an acidic lumen. This luminal pH is regulated and maintained by a balance between proton pumping by vacuolar-ATPase (v-ATPase), proton conductance, and intrinsic proton leaks [23–28]. Membrane bound vesicles in the endocytic pathway (early endosome, recycling endosome, late endosome, and lysosomes) and the biosynthetic secretory pathway (Golgi apparatus and secretory vesicles) all display varying degrees of acidity (Fig. 1). The acidic pH enables efficient sorting and trafficking of newly synthesized and internalized molecules and/or membranes to their intended destinations for proper processing and function [23,29,30].

The endolysosome system is a dynamic interconnected network with morphological and functional heterogeneity, and this system exhibits complex interactions with other organelles. Extracellular macromolecules and membrane components are up-taken into endosomes by a variety of endocytic pathways and can either be trafficked through early endosomes to recycling endosomes, which mediates receptor recycling to plasma membrane or Golgi apparatus, or can transition to late endosomes and fusion with lysosomes [26,31–33]. As mentioned above, a hallmark feature of the endolysosome system is its acidic luminal pH [25–28] that is critical for the activity of up to 60 different pH sensitive hydrolytic enzymes including proteases, lipases and nucleases [33].

Early endosomes act as a major sorting station and their acidic nature enables internalized ligands to be dissociated from the endocytosed cell surface membrane receptors to which they were bound. The receptors unbound with ligand can then be recycled back to the cell surface or can traffic to Golgi apparatus via recycling endosomes; dissociated ligands are transported through late endosomes to lysosomes for degradation. The maturation of early endosomes to late endosomes is characterized by an increased number of intralumenal vesicles and the formation of multivesicular bodies; a process requiring an acidic intraluminal pH [34]. The acidic pH and acidic hydrolases are critical for degradation of cytosolic proteins and obsolete organelles via the formation of autophagosomes followed by fusion with late endosomes/lysosomes; the so-called “self-eating” or autophagy that is essential for cell homeostasis and development [35,36]. In addition to the above, endolysosomes control a range of physiological functions including antigen processing, membrane repair, nutrient sensing, and ion homeostasis [37–40].

Endolysosome pH can be disturbed by many factors. Classical lysosomotropic weak bases such as NH₄Cl, CQ, and methylamine [41] as well as many FDA approved drugs (possibly weak bases) including antiretroviral drugs [42] and anti-cancer drugs [43] tend to accumulate in acidic endolysosomes and neutralize their pH. Carboxylic ionophores, such as monensin and nigericin, can cause electroneutral exchange of protons across the membrane against other monovalent cations [44]. Inhibitors of v-ATPase (bafilomycin and concanamycin) rapidly elevate the pH in acidic organelles [45]. Abnormal accumulation in endolysosomes of low-density lipoprotein (LDL) cholesterol [46], HIV-1 viral proteins such as Tat and gp120 [47,48], and intracellular pathogens including Mycobacterium tuberculosis [49,50], urapathogenic E. coli [51], and coxiella burnetii [52] also cause endolysosome de-acidification. Further, endolysosome pH is affected by alterations in ion permeability (K⁺, Cl⁻, Ca²⁺, Fe²⁺, Zn²⁺) across endolysosome membranes, changes in endolysosome membrane potential, cellular nutritional status, and cellular signaling [53–58]. Significantly, defective endolysosome pH regulation has been implicated in a growing number of human diseases including osteopetrosis, renal tubular acidosis, cutis laxa [59], Dent’s disease [60], Christianson syndrome, autism and attention deficit hyperactivity disorder [61]. Furthermore, it is being increasingly linked to cellular aging, synaptic dysfunction, cancer, and neurodegenerative disorders including Alzheimer’s and Parkinson’s disease [53] and HIV-associated neurocognitive disorders [47,62–65].

CQ and its analogs HCQ (Fig. 1) are concentrated in acidic endolysosomes [66–68] where they neutralize endolysosome pH [20,55], induce markedly enlargement of endolysosomes [69,70], change the positioning of endolysosomes from perinuclear to the periphery of cells [48,71], and lead to lysosome membrane permeabilization [54,72,73]. CQ-induced endolysosome de-acidification results in the accumulation and aggregation of undegraded substrates and atypical cleavages that lead to generation of toxic intermediates [42,74]. CQ-induced lysosomal membrane permeabilization leads to the translocation of
lysosomal contents (eg. cathepsins) to the cytoplasm and to the induction of mitochondria damage and cell death [75,76]. CQ-induced endolysosome de-acidification also impairs vesicular fusion and inhibits autophagic flux by decreasing autophagosome-lysosome fusion [70,77]. In addition, CQ enhances lysosome exocytosis [72,78] and the release of exosomes [79]. However, CQ does not affect endocytosis [70].

Golgi apparatus helps process, sort and traffic proteins and lipids destined for secretion, membranes, and organelles. Sub-compartments of Golgi are mildly acidic; pH values range from 6.7 at cis-Golgi to 6.0 at trans-Golgi [29]. Secretory vesicles (constitutive or regulated) are more acidic; luminal pH ranges from 5.2 to 5.7 [23,24]. Consistent with the view that an acidic environment is critical for the processing of proteins and lipids, deacidification of Golgi results in defects in post-translational modifications and processing of secreted proteins. For example, glycosylation is pH-sensitive [80] and an increase of 0.2 pH units results in decreased glycosylation [81]. In terms of sorting and trafficking of proteins and lipids, deacidification impaired anterograde transport from Golgi to secretory vesicles [82], retrograde transport from Golgi back to the endoplasmic reticulum (ER) [83], the delivery of lysosomal hydrolases to lysosomes via mannose-6-phosphate receptor (M6PR) [78,84], the integrity of Golgi itself [85,86], and the sorting and proteolytic maturation of prohormones in secretory granules [87]. Thus, it is not surprising that defective Golgi pH regulation has been implicated in a number of human diseases including autosomal recessive Cutis Laxa type II [88] and multigenerational non-syndromic intellectual disability [89].

Similar to endolysosomes, CQ and HCQ (Fig. 1) are concentrated in and neutralize the pH of acidic Golgi [29], and induce marked dilatation of the Golgi cisternae [90]. Functionally, CQ-induced de-acidification results in glycosylation deficits [81], defects in the formation of functional transport vesicles, and the inability of budding vesicles to pinch off and form functional transport vesicles [91,92]. CQ also changed distribution patterns of mannose-6-phosphate receptors and decreased the delivery of lysosomal enzymes into lysosomes via mannos-6-phosphate receptors [93]; the latter process might be responsible for CQ-induced changes in lysosome exocytosis [72,78]. Furthermore, CQ-induced de-acidification also leads to deficits in sorting and proteolytic maturation of the prohormones pro-somatostatin [94], adrenocorticotropic hormone (ACTH) [95], and pro-insulin [96].

2.3. Summary of cell biology concerns

As diprotic weak bases, CQ and HCQ are taken up by cells and trapped in acidic organelles; they are only extruded by exocytosis and/or through the multidrug resistance protein p-glycoprotein [97-99]. Through their ability to deacidify acidic organelles along endocytic and biosynthetic secretory pathways, CQ and HCQ disturb many key aspects of cell biology including organellar biology and inter-organellar signaling, many of which are linked to their antiviral and immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects. Decades of clinical usage have shown that CQ and HCQ are relatively safe drugs. They are even commonly used as immunosuppressive effects.
the fusion of viral membranes with endosome membranes and the release of viral genomic content into the cytoplasm [121]. As such, endolysosome de-aciddification with a v-ATPase inhibitor [122] or CQ [123,124] has been used frequently to inhibit cellular entry of enveloped virus in vitro. Once replicated in the cytoplasm, some viruses are packaged in trans-Golgi network where low pH facilitates the maturation of the virus. CQ, by de-aciddifying Golgi, impairs the maturation of viruses and decreases viral infection, in part, by increasing the accumulation of, for example, non-infectious herpes simplex virus 1 particles [125], HIV-1 [126] and flavivirus [126–129]. Currently it is not clear whether CQ affects membrane invagination and viral packing into the trans-Golgi network or the extracellular release of mature virus.

Given the above findings, it is not surprising that CQ and HCQ are being tested for their possible effectiveness against SARS-CoV-2. Preliminary in vitro studies have shown that both CQ [9] and HCQ [10,11] exhibit antiviral effects against SARS-CoV-2. Although the underlying mechanisms are not fully understood, it is possible that endolysosome de-aciddification by CQ (Fig. 2) may block pH-dependent, furin- or cathepsin L-mediated cleavage of the spike envelope protein that facilitates viral envelope fusion with endosome membranes [115,116,118,119]. Further, CQ-induced Golgi de-aciddification may result in decreased expression levels of ACE2. Such mechanisms have been implicated previously with SARS-CoV; CQ decreased the binding of SARS-CoV spike protein with ACE2 [8] and CQ-induced Golgi de-aciddification (Fig. 2) affected post-translational modifications including the proteolysis and glycosylation of SARS-CoV virions [8].

In the context of COVID-19, CQ and HCQ are often used in combination with antivirals and other drugs such as azithromycin and zinc [130,131]. By de-aciddifying acidic organelles, CQ and CQ could alter the cellular distribution of these drugs. Azithromycin, a macrolide antibiotic, has been used against Zika [132] and Ebola viruses [133]. In vitro evidence indicates that HCQ and azithromycin show synergistic effects on inhibiting SARS-CoV-2 [130]. As a weak base, azithromycin (pKa = 8.5) also tends to accumulate in acidic environments [134]. By de-aciddifying acidic organelles, CQ and HCQ could prevent the accumulation of azithromycin in acidic organelles and increase its concentrations in cytosol where azithromycin exerts inhibitory effects on viral replication [130]. On the other hand, such CQ- and HCQ-induced cellular redistributions of azithromycin may exaggerate its QT prolongation effects [135] as observed in the context of COVID-19 [136]. In vitro evidence indicates that zinc inhibits SARS-CoV [137]. In a preprint paper [131], the authors reason that CQ, as a zinc ionophore [138], could increase cytosolic concentrations of zinc to inhibit RNA-dependent RNA polymerase. This is contrary to the original observation that CQ is a zinc ionophore [138], in which chloroquine was observed as a zinc ionophore that targets zinc to lysosomes. Nonetheless, zinc has been shown to inhibit the activity of furin [139] and cathepsins [140]. Interestingly, the activity of furin is also pH-dependent; furin’s optimum pH is 6.0 [141]. Thus, in addition to its de-aciddifying effects, CQ-induced accumulation of zinc in endolysosomes could further inhibit activities of furin and cathepsins that are responsible for cleavage of SARS-CoV spike proteins and viral entry.

De-aciddifying acidic organelles may not be the only mechanism whereby CQ and HCQ interfere with the biology of SARS-CoV2. In human epithelial lung cells, CQ inhibits the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and inhibits the release of human coronavirus from infected cells [142]. Thus, CQ and HCQ may exert its effects on SARS-CoV-2 via altering protein kinase activities.

3.1. Concerns about the possible antiviral effects of CQ and HCQ

Currently, there exist very limited evidence supporting the use of CQ and HCQ against COVID-19. First, almost all in vitro studies reported to date were conducted in epithelioid cells derived from the kidney of African green monkeys (Vero E6 cells) or human hepatomas (Huh7 cells) [129,143,144]. Vero E6 cells are extremely permissiveness for viral replication including coronaviruses, which is due in part to genetic defects in interferon production [145,146] and defects in innate antiviral responses [147]. Huh7 cells are also highly permissive for virus replication due to defective retinoic acid-inducible gene I signaling, impaired interferon signaling, and defects in innate antiviral responses [148,149]. Thus, it is not clear how finding from these cells are translatable to human clinical trials. Second, CQ and HCQ change the pH of endolysosomes and Golgi and cause multiple morphological and functional changes to these organelles including swelling and altered exocytotic release of virus. These changes may result in the formation of intracellular SARS-CoV-2 reservoirs capable of being re-activated when pH normalizes. Most recently (June 23, 2020), several clinical studies have reported on the favorable use of CQ and HCQ for the treatment of COVID-19 [150–159]. However, other studies have reported no significant beneficial effects and some detrimental effects [160–162]. Multiple clinical trials testing the possible effectiveness of CQ and HCQ against COVID-19 are ongoing [163], the results of these studies will be important as more people require treatments for COVID-19.

4. Immunomodulatory properties of CQ and HCQ

The immunomodulatory properties of CQ and HCQ have long been recognized and these drugs continue to be used clinically for the treatment of rheumatoid arthritis, systemic lupus erythematosus and other inflammatory rheumatic diseases [164]. Although their mechanisms of action remain under investigation, the immunomodulatory effects are likely due to their accumulation in and de-aciddification of acidic compartments; endolysosomes and Golgi apparatus of immune cells. The importance of CQ- and HCQ-induced de-aciddification as an important mediator in immune modulation may be due to impaired maturation of lysosomes and blockade of fusions between autophagosomes and lysosomes. Indeed, lysosomal degradation of endocytosed or autophagocyted proteins affects antigen processing and MHC class II presentation [165,166]. This is consistent with findings that CQ and HCQ inhibit MHC class II expression, antigen presentation and immune activation [167]. Further, RNA and DNA binding to toll-like receptor 7 (TLR7) and TLR9 in endosomes results in TLR signaling activation and production of pro-inflammatory cytokines [168]. Thus, CQ- and HCQ-induced endolysosome de-aciddification could interfere with ligand binding with TLRs and inhibition of TLR signaling and the production of pro-inflammatory cytokines [164,169].

CQ and HCQ also affect immunoregulation by inhibiting the release of various pro-inflammatory cytokines such as IL-1, IFNα and TNFα [98,170]. Most cytokines require conversion to soluble more active mature forms and CQ and HCQ by deacidifying Golgi block such conversion of, for example, pro-TNFα to its soluble mature form [170,171]. Two pathways for cytokine secretion also require acidic vesicles. The classical pathway for cytokine secretion (Fig. 3) involves direct transport along trans-Golgi secretory pathways to the extracellular space (eg. IL-10) or indirectly though recycling endosomes to the extracellular space (egs. TNFα, IL-6, and IL-10) [172]. The non-classical pathway for cytokine secretion (Fig. 3) involves transport of cytokines (eg. IL-1β) into autophagosomes and fusion with endosomes to form amphisomes before being released into the extracellular space [173,174]. By deacidifying endolysosomes and Golgi, CQ and HCQ could reduce the secretion of, for example, the proinflammatory cytokines TNF-α, IL-1β and IL-6 [98,170]. Because elevated systemic IL-6 levels in patients with COVID-19 have been implicated as a relevant parameter in predicting severity of disease and the need for intensive care [175], CQ and HCQ may alter the course of COVID-19 by controlling IL-6 and delaying or preventing the development of critical stages of the disease. In addition, CQ could decrease the surface expression of TNFα receptors and thereby inhibit receptor-mediated TNFα signaling [176].
a potential con- of any commercial or financial relationships that could be construed as a conflict of interest.

5. Summary

The FDA issued an Emergency Use Authorization for the use of CQ and HCQ against COVID-19. The clinical effectiveness of CQ and HCQ against COVID-19 is being studied in over a dozen controlled clinical trials. However, the safety of using these drugs may be questioned especially if the proinflammatory cytokine TNF-α, IL-1β and IL-6.

Declaration of Competing Interest

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

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