Chloroquine and Hydroxychloroquine in the Era of COVID-19: A Mini-Review

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

ABSTRACT

Coronavirus disease of 2019 (COVID-19) has severely affected global health, and caused a significant health burden worldwide in all, the patients, and healthy people. Globally, 17 August 2021, there have been 207,784,507 confirmed cases of COVID-19, including 4,370,424 deaths reported to WHO. As of 16 August 2021, a total of 4,462,336,040 vaccine doses have been administered. Many risk factors, such as older adults, people with medical conditions and pregnant people and recently pregnant people are at an increased risk for severe illness from COVID-19 when compared to non-pregnant people. CQ and HCQ also used in the management of rheumatism, joint inflammation, systemic lupus and showed positive and promising results in the primary research for COVID-19 management but, it’s still confusing whether to use them in patients infected with COVID-19 or it is better to avoid them. Although these drugs, chloroquine (CQ) and hydroxychloroquine (HCQ), which have been tried for the treatment of this disease, COVID-19 vaccines can help end the pandemic. In this review we aim to evaluate the evidence regarding the

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efficacy and safety of CQ and HCQ used against viral infection caused by COVID-19. Our hope is to shed light on CQ and HCQ as a management modality in the era of COVID-19. We can't do without drug therapy as a mainstream till antiviral agents and effective vaccines would be available.

Keywords: COVID-19; chloroquine; hydroxychloroquine; treatment; review.

1. INTRODUCTION

A novel coronavirus (nCoV), also named “SARS-CoV-2”, was declared by the World Health Organization (WHO) in December 2019 to be responsible for the outbreak of COVID-19. There have been 207,784,507 confirmed cases of COVID-19, comprising 4,370,424 deaths, reported to WHO 16 August 2021 [1]. Coronaviridae is the main family to which Coronaviruses belong. Coronavirinae and Torovirinae are two subfamilies divided from the main family. Coronavirinae is also subdivided into four genera: Alpha, Beta, Gamma and Delta-coronavirus [2]. SARS-CoV-2 is closely related to the beta-coronaviruses. Like other coronaviruses, SARS-CoV-2 genome is positive, single-stranded RNA with a 5'-cap, 3'-UTR poly(A) tail [3]. At the beginning, case fatality rate of the virus was measured to be 2%, but in some countries, ranged from 4 to 9%. Meanwhile, the actual fatality rate was 1% after adjustment for asymptomatic cases. Now, the major fears of COVID-19 are the rapid transmission and the substantial proportion of asymptomatic people who responsible for about 40-50% of transmission [4].

Many efforts, including both pharmacological and non-pharmacological interventions, are being done to fight against this virus. In a trial to find a potential pharmacologic substance that may be useful to protect against that virus and/or treat COVID-19 patients. Clinicians have considered chloroquine (CQ) and hydroxychloroquine (HCQ) as a treatment regimen [4]. Many rationales supported the selection of this regimen. For example:

A) The great success of this regimen to cure against SARS-CoV-1.
B) Many experiment proved the ability of this drug to prevent in vitro viral replication [4].

Since this time, the world has divided into one with while the others against this regime. Currently more than one billion people are in lockdown in their homes, flights have been cancelled, and the global transportation system has become paralyzed worldwide in response to the highly contagious nature of the COVID-19. Till now, there is no effective, proved pharmacological treatment for it. In-vitro studies have suggested that chloroquine and hydroxychloroquine, an immunomodulant drugs traditionally used to treat malaria, are effective in reducing viral replication in other infections, including the SARS-associated coronavirus (CoV) and MERS-CoV [5,6]. In this review, we aim to investigate indirect epidemiologic evidence of the antiviral characteristics of hydroxychloroquine and chloroquine in the treatment of COVID-19 infection.

2. STRUCTURE OF THE SARS-COV-2 VIRUS

SARS-CoV-2 is round or elliptic in shape with pleomorphic structure and has small size, ranging from 60 to 140 nm in diameter. The outer morphology of SARS-CoV-2 has multiple nucleoproteins, membranous proteins and polyproteins-like spike glycoproteins S [7]. The latter has homo-trimers that project from the outer surface of the virus’s, resulting in a halo-like shape.

The virus is formed of 6 open-reading frames, common coronavirus and some other additional genes [8]. SARS-CoV-2 RNA genome comprises 29,891 nucleotides, composing around 9860 amino acids. Its particles comprise five major structural proteins namely: glycoprotein spikes (S), an envelope protein (E), matrix protein (M), and nucleocapsid (N) protein [9]. Through glycoprotein spikes the virus attach to host cell receptors, according to the receptor-binding domain (RBD). Once attached to the host cell receptor, it cleavages into two subunits namely, N-terminal S1 and C-terminal S2 subunit regions by the host proteases enzyme [9]. S1 subunit contains a signal peptide and a RBD. While S2 subunit contains fusion peptide (FP), heptad repeat (HR) peptides, transmembrane domain (TM), and a cytoplasmic domain [9]. The M glycoprotein gives the virus its shape. It is a pre-glycosylated M polypeptides [10]. Envelope protein (E) is polypeptide and is the integral membrane protein [9,10]. Although we do not
completely recognize its origin, the analysis of its genomic propose the possibility it developed from a species subsisted in bats [11,12]. This virus has cross transportation property, animal to animal, animal to human and human to human [13].

3. CHEMICAL COMPOSITIONS AND SOURCES OF CQ AND HCQ

Hydroxychloroquine is a disease-modifying anti-rheumatic drug (DMARD) with a chemical structure very similar to that of chloroquine [14-16]. Both are absorbed from the upper intestinal tract [17]. Systemic modifications are added to quinine, which is a plant alkaloid and quinoline containing compound, to produce CQ. HCQ is a derivative of CQ and synthesized from it by adding a hydroxyl group to CQ [4]. The toxicity of CQ in animals is believed to be three times more than HCQ [18]. Also, HCQ is used widely to treat auto-immune diseases such as, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [19].

4. MECHANISM OF ACTION

Chloroquine (CQ) and hydroxychloroquine (HCQ) were originally used to cure patients with rheumatoid arthritis (RA) and those diseased with lupus (SLE) [20]. Both drugs have the same mechanism of action and adverse effect. And all of which are well known and reported [21-23]. A short time ago, CQ and it’s derivative HCQ were discovered to treat many viral infections, e.g. hepatitis A, AIDS and Borna disease [5]. Many studies say CQ and HCQ may have an impact on COVID-19 by two diverse mechanisms; increasing the pH of endosomes, regulating lysosomes and improving immunity [24,25]. For COVID-19, the modulation of pH could be the commonest process, when in the unprotonated form, CQ/HCQ can diffuse passively across cellular membranes, where it is protonated and trapped in endosomes/lysosomes, resulting in alkalinization of the acidic compartments. Due to this alkaline media, the virus cannot enter the host cell. This entry was originally facilitated by spike proteins in the host cell (ACE2). For spike protein priming, this procedure involves the serine protease TMPRSS2, whereas TMPRSS2 priming uses endosomal cysteine proteases cathepsin B/L, which requires a low pH environment [26].

HCQ/CQ also inhibit glycosylation of angiotensin converting enzyme-2, to which the SARS-Cov-2 is attached [27]. HCQ/CQ downregulate the toll like receptor (TLR) on activated immune cells and block TLR signal transduction and prohibit inflammatory factors secretion, such as IL-6 [28,29]. CQ/HCQ may operate as an anti-thrombotic drug by modulating endosome/lysosome function, preventing hypoxia, which is frequent in acute respiratory distress syndrome (ARDS). All this serve finally and lead to a good vascular state [30-32]. Though, coronavirus could attach the host cells through different non-endosomal pathway which could not be blocked by HCQ/CQ [33].

5. ADVERSE EFFECTS

Indeed, both CQ and HCQ cause severe adverse effects when prescribed in a high dose. This is mediated mainly by modulation of cellular excitability and action potential of excitable cells such as, neurons and cardiac myocytes [19].

5.1 Ocular Manifestations

Ocular manifestations like diplopia, decreased visual acuity, retinopathy, retinal detachment, and bilateral visual loss were found to be the most dangerous adverse effects caused by high doses of CQ [34]. Also, it can lead to maculopathies and macular degeneration which may be irreversible.

5.2 Psychiatric Manifestation

High dosage CQ causes major psychiatric issues as paranoia, hallucinations, and suicidal attempts [35]. Intramuscular CQ has proved to cause life-threatening hypotension [36].

5.3 Cardiac Manifestation

On the heart, both proved to cause a decrease of myocardial activity and lead to manifest cardiac electrical conduction and QT interval elongation [36]. Clinical trials found that the mean prolongation of QT interval was about 35 [28–43] ms and QT interval exceed 500 ms during treatment with chloroquine in more than 20% percent of COVID-19 patients [37]. So, it should be taken in consideration when managing COVID-19, as in severe cases of this disease, there is increased cardiac mortality due to ARDS, and direct cardiac injury [38]. There are other adverse effects experienced such as metabolic abnormalities (hypokalemia, magnesemia and calcemia), pruritus, photosensitivity, seizures,
6. THE CQ/HCQ EFFICACY IN LINE WITH THE PATHOGENESIS OF COVID-19

Indeed, there is a great conflict regarding the results of in vitro studies about the CQ/HCQ efficacy and the results of clinical trials has been unsatisfying. While several reports have reported a great improvement in efficacy when administrated alone or with a concurrent use of other antiviral agents like, azithromycin or zinc [28,42], also a limited benefits were reported as a prophylaxis or treatment [43,44]. Probably, there is difference between in vitro and in vivo Ca²⁺ dynamics. Also, Ca²⁺ homeostasis changes during the progression of the disease in COVID19 patient from prodromal, to the mild and severe phases. During these phases COVID-19 become highly heterogenous, involving respiratory and renal systems leading to hypoxia and impaired homeostasis. Other factors; such as PH, temperature, oxygen and ion components are well managed, and this cannot be achieved when pulmonary, renal, and circulatory function are impaired [45]. It’s worth noting that a low plasma pH relates to a low concentration of CQ/HCQ in endosomes/lysosomes and a higher risk of blood coagulation [46,47]. It’s worth noting that when Zn²⁺ and Azithromycin are utilized as adjuvants in conjunction with CQ/HCQ, we can produce a synergistic effect [48-50]. As a result, a plasma Zn²⁺ concentration test in conjunction with a CQ/HCQ concentration assay is critical in clinical practice. There is no pharmacokinetic interaction between azithromycin and chloroquine, other from the CQ/Zn²⁺ synergistic effects [51].

7. CALCIUM HOMEOSTASIS AND THE RELATION TO ANTIVIRAL AGENTS

Calcium channels and intra-cellular calcium are critical targets for RNA transcription, duplication or budding [52,53]. CQ/HCQ regulate calcium channels in atrial trabeculae, B lymphocyte [53,54] and CD4⁺thymocytes as they have an antagonistic effect [54]. It was proved that CQ inhibits IP₃R mediated Ca²⁺ release from intra-cellular stores and (TRPC3) channel mediated Ca²⁺ influx in CD4⁺ T cells. Also, we found that Ca²⁺ in cortical neurons that is arisen from human induced pluripotent stem cells (iPSCs) is diminished by chloroquine. Hence, we concluded that chloroquine could modulate intracellular Ca²⁺ by diminution extracellular Ca²⁺ entry and release of intracellular store-operated calcium (SOC). As a result, CQ/HCQ may inhibit viral pathogenicity by interfering with cytosolic Ca²⁺, which is essential for viral lifecycles in vulnerable cells such as airway epithelial cells, pulmonary capillary endothelial cells, and other cells [44,55]. Involvement of Ca²⁺ in either viral replication or budding has been demonstrated in several viruses, as rotavirus, polyomavirus and Hepatitis A or B virus [56-58]. Furthermore, the SARS-CoV spike protein has been shown to cause extracellular Ca²⁺ influx +, which causes the Ca²⁺-dependent protein kinase C alpha to be activated [59]. The downstream ERK/NF-kappa B pathway, which is necessary for COX-2 protein expression in SARS-associated coronavirus triggered cyclooxygenase-2, is regulated by PKC alpha (COX-2). As a result, by reducing Ca²⁺ influx into host cells, CQ/HCQ may lessen inflammation. CQ/HCQ may also directly control virulence by modulating coronavirus envelope (E) protein Ca²⁺ transport. The SARS-CoV virus's E protein is a viroporin, which allows Ca²⁺ and other cations to pass through. This is a crucial step in the production of pro-inflammatory cytokines such as IL-1. SARS E protein can even form a protein-lipid channel in the Golgi membrane, through which Ca²⁺ mediates pH and E protein pore selectivity. As a result, CQ/HCQ may alter immune response by limiting Ca²⁺ influx through viroporin and reducing SARS-CoV-2-induced, exacerbated pro-inflammation [60,61]. CQ/HCQ may also operate as an immunomodulator by inhibiting Ca²⁺-dependent calcineurin activity [62]. The suppression of calcineurin limits nuclear factor translocation in activated T cells, which prevents cytokine transcription. These findings imply that cytosolic Ca²⁺ modification is a fundamental mechanism through which CQ/HCQ works as a broad-spectrum antiviral [52,63,64]. Given the foregoing facts, as well as the tight phylogenetic relationship between SARS-CoV and SARS-CoV-2, calcium targeting is likely to be a portion of COVID-19’s CQ/HCQ effects.

8. EVIDENCE OF USING CQ AND HCQ TO MANAGE COVID-19 PATIENTS AND RECOMMENDATIONS

Till now, there are very limited data proving the use of CQ and HCQ for the management or prevention of COVID-19. A pilot study in China [65] on 30 confirmed patients with COVID-19 was done by dividing the patients into two groups, group a and group b. Group a received paranoia, hallucinations, vomiting, diarrhea, and retinopathy [39-41].
the standard treatment and group b received standard treatment plus hydroxychloroquine (400 mg for 5 days). The results were noted to be identical in both groups. By day 7, more patients in the group on standard treatment alone (93.3%) had a negative throat swab compared to those taking the HCQ (86.7%). Also, it was notable that patient on HCQ showed marked reduction in pneumonia (on CT scan). One patient in the HCQ group developed severe disease [65]. There were some limitations in this study such as small sample size.

On April 21, 2020, a reasonably large US study (n=368) of veterans hospitalized with COVID-19 revealed no evidence that using HCQ, with or without azithromycin, lowered the incidence of mechanical ventilation in COVID-19 patients. In patients treated alone with HCQ, there was a link to higher overall mortality [66]. This was a retrospective, non-randomized study that has not yet been peer reviewed. A small population of mostly African American males over the age of 65 was also a constraint. The authors could not rule out the possibility of selection bias or residual confounders after adjusting for variables such as comorbidities and concomitant drugs [66]. Many guidelines support the usage of CQ or HCQ for the management of COVID19 patients. Belgian guidelines support HCQ for severe disease and recommend it for mild to moderate disease; Chinese guidelines recommend CQ for hospitalized patients, while later revisions have voiced concern about dosing and special patient categories [67,68]; Italian guidelines recommend early usage of CQ or HCQ [69]. The WHO, and Public Health England are yet to recommend CQ or HCQ for treatment.

9. RECENT UPDATES REGARDING MANAGEMENT OF COVID-19

It is well known that understanding the interactions between the target and the ligand of the COVID-19 is the most crucial and difficult step in discovering drug for COVID-19. CoViTris2020 and ChloViD2020 were discovered to have proteins-inhibiting characteristics on SARS-CoV-2. The molecules CoViTris2020 and ChloViD2020 have been successfully reevaluated, repurposed, and reported as very promising hit molecules (they could also be considered the first extremely potent anticonoraviral-2 inhibitors). "Coronavirus-2 Killers" are polyphenolic 1,3,4-oxadiazole compounds having broad antiviral activity. SARS-CoV-2 enzymes are successfully inhibited by a multitarget and extremely powerful inhibitor. As a result, both compounds are two of the earliest recognized promising compounds. Potential medicines under study for the effective and comprehensive treatment of COVID-19 [70,71].

10. CONCLUSION

Hydroxychloroquine and chloroquine don’t lower the incidence of SARS-CoV-2 transmission than normal regimens. Adverse events are high; some of the most typically documented adverse effects of CQ/HCQ are likely to obstruct successful treatment of COVID-19 patients. Thought, no treatment-related serious adverse events were reported. As a result, until sufficiently powered randomized controlled trials (RCTs) give more evidence on the efficacy and safety of CQ/HCQ use in the treatment of COVID-19 patients, it is critical to balance the possible advantages of these drugs against the potential hazards. Furthermore, long-term, e.g., 3–6 months post-therapy, side effects of CQ/HCQ use in COVID-19, such as cardiomyopathy, muscle weakness, anxiety, insomnia, and gastrointestinal issues, should be evaluated in clinical trials. Off-label usage of CQ/HCQ should preferably be reserved only for COVID-19 patients treated in the context of clinical studies until evidence from RCTs becomes available, to improve our knowledge of safety and efficacy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Covino M, Sandroni C, Santoro M, Sabia L, Simeoni B, Bocci MG, et al. Predicting intensive care unit admission and death for COVID-19 patients in the emergency department using early warning scores. Resuscitation. 2020;156:84-91.
2. Phan M, Tue N, Anh P, Baker S, Kellam P, Cotten M. Identification and characterization of Coronaviridae genomes from Vietnamese bats and rats based on
conserved protein domains. Virus Evolution. 2018;4.
3. Abduljalil JM, Abduljalil BM. Epidemiology, genome, and clinical features of the pandemic SARS-CoV-2: A recent view. New microbes and new infections. 2020;35:100672.
4. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell discovery. 2020;6(1):16.
5. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? The Lancet Infectious diseases. 2003;3(11):722-7.
6. Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. International journal of antimicrobial agents. 2020;55(3):105923.
7. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science (New York, NY). 2020;367(6483):1260-3.
8. Zhou P, Yang XL, Wang XD, Hu B, Zhang L, Zhang W, et al. Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;588(7836):E6.
9. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging microbes & infections. 2020;9(1):221-36.
10. Chen J. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes and infection. 2020;22(2):69-71.
11. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet (London, England). 2020;395(10244):565-74.
12. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Journal of advanced research. 2020;24:91-8.
13. Meo SA, Alhowikan AM, Al-Khlaiwi T, Meo IM, Halepoto DM, Iqbal M, et al. Novel coronavirus 2019-nCoV: Prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. European review for medical and pharmacological sciences. 2020;24(4):2012-9.
14. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell discovery. 2020;6:16.
15. Benjamin O, Bansal P, Goyal A, Lappin SL. Disease modifying anti-rheumatic drugs (DMARD). StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2021, StatPearls Publishing LLC.; 2021.
16. Zhou D, Dai SM, Tong Q. COVID-19: A recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. The Journal of antimicrobial chemotherapy. 2020;75(7):1667-70.
17. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: Implications for rheumatology. Nature reviews Rheumatology. 2020;16(3):155-66.
18. McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. The American journal of medicine. 1983;75(1a):11-8.
19. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. International journal of antimicrobial agents. 2020;55(4):105932.
20. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology. 2015;23(5):231-69.
21. Semb SO, Jacobsen D. [Chloroquine poisoning]. Tidsskrift for den Norske laegeforening : Tidsskrift for praktisk medicin, ny raekke. 1996;116(4):478-80.
22. Maxwell NM, Nevin RL, Stahl S, Block J, Shugarts S, Wu AH, et al. Prolonged neuropsychiatric effects following management of chloroquine intoxication with psychotropic polypharmacy. Clinical case reports. 2015;3(6):379-87.
23. Abena PM, Decloedt EH, Bottleau E, Suleman F, Adejumo P, Sam-Agudu NA, et al. Chloroquine and hydroxychloroquine
for the prevention or treatment of COVID-19 in Africa: Caution for inappropriate off-label use in healthcare settings. The American journal of tropical medicine and hygiene. 2020;102(6):1184-8.

25. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronavirus - drug discovery and therapeutic options. Nature reviews Drug discovery. 2016;15(5):327-47.

26. Tripathy S, Dassarma B, Roy S, Chabalala H, Matsabisa MG. A review on possible modes of action of chloroquine/hydroxychloroquine: repurposing against SAR-CoV-2 (COVID-19) pandemic. International journal of antimicrobial agents. 2020;56(2):106028.

27. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271-80.e8.

28. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005;2:69.

29. Yu B, Li C, Chen P, Zhou N, Wang L, Li J, et al. Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19. Science China Life sciences. 2020;63(10):1515-21.

30. Alia E, Grant-Kels JM. Does hydroxychloroquine combat COVID-19? A timeline of evidence. J Am Acad Dermatol. 2020;83(1):e33-e4.

31. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endotheliitis, thrombosis, and angiogenesis in COVID-19. The New England journal of medicine. 2020;383(2):120-8.

32. Jung H, Bobba R, Su J, Shariat-Sarabi Z, Gladman DD, Urowitz M, et al. The protective effect of antimalarial drugs on thrombovascular events in systemic lupus erythematosus. Arthritis and rheumatism. 2010;62(3):863-8.

33. Maes H, Kuchnio A, Carmeliet P, Agostinis P. Chloroquine anticancer activity is mediated by autophagy-independent effects on the tumor vasculature. Molecular & cellular oncology. 2016;3(1):e970097.

34. Zumla A, Chan JFW, Azhar EI, Hui DSC, Yuen K-Y. Coronaviruses — drug discovery and therapeutic options. Nature Reviews Drug Discovery. 2016;15(5):327-47.

35. Kamat S, Kumari M. Repurposing chloroquine against multiple diseases with special attention to SARS-CoV-2 and associated toxicity. 2021;12(339).

36. Kumar R, Sharma A, Srivastava JK, Siddiqi MH, Uddin MS, Aleya L. Hydroxychloroquine in COVID-19: therapeutic promises, current status, and environmental implications. Environ Sci Pollut Res Int. 2021;28(30):40431-44.

37. White NJ. Cardiotoxicity of antimalarial drugs. The Lancet Infectious diseases. 2007;7(8):549-58.

38. van den Broek MPH, Möhlmann JE, Abeln BGS, Liebregts M, van Dijk VF, van de Garde EMW. Chloroquine-induced QT prolongation in COVID-19 patients. Netherlands heart journal : Monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation. 2020;28(7-8):406-9.

39. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovascular research. 2020;116(6):10097-100.

40. Juurlink DN. Safety considerations with chloroquine, hydroxychloroquine and azithromycin in the management of SARS-CoV-2 infection. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2020;192(17):E450-e3.

41. [Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia]. Zhonghua jie he huxi za zhi = Chinese journal of tuberculosis and respiratory diseases. 2020;43(3):185-8.

42. Rajeshkumar NV, Yabuuchi S, Pai SG, Mastra A, Hidalgo M, Dang CV. Fatal toxicity of chloroquine or hydroxychloroquine with metformin in mice. bioRxiv. 2020:2020.03.31.018556.

43. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. International journal of antimicrobial agents. 2020;56(1):105949.

44. Skipper CP, Pastick KA, Engen NW, Bangdiwala AS, Absi M, Lofgren SM, et al. Hydroxychloroquine in Nonhospitalized
Alfawaz and Alfawaz, JPRI, 33(44A): 228-236, 2021; Article no.JPRI.73599

Adults With Early COVID-19: A Randomized Trial. Ann Intern Med. 2020;173(8):623-31.

45. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. Jama. 2020;324(8):782-93.

46. Takahashi T, Suzuki T. Low-pH Stability of Influenza A virus sialidase contributing to virus replication and pandemic. Biological & pharmaceutical bulletin. 2015;38(6):817-26.

47. Crowell JW, Houston B. Effect of acidity on blood coagulation. The American journal of physiology. 1961;201:379-82.

48. Warhurst DC, Craig JC, Adaqu IS, Guy RK, Madrid PB, Fivelman QL. Activity of piperazine and other 4-aminoquinoline antiplasmodal drugs against chloroquine-sensitive and resistant blood-stages of Plasmodium falciparum. Role of beta-haematin inhibition and drug concentration in vacuolar water- and lipid-phases. Biochemical pharmacology. 2007;73(12):1910-26.

49. Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, Sandoval-Espinosa C, et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(50):14408-13.

50. Schögl A, Kopf BS, Edwards MR, Johnston SL, Casaluta C, Kieninger E, et al. Novel antiviral properties of azithromycin in cystic fibrosis airway epithelial cells. The European respiratory journal. 2015;45(2):428-39.

51. te Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS pathogens. 2010;6(11): e1001176.

52. Cook JA, Randinisitis EJ, Bramson CR, Wesche DL. Lack of a pharmacokinetic interaction between azithromycin and chloroquine. The American journal of tropical medicine and hygiene. 2006;74(3):407-12.

53. Clark KB, Eisenstein EM. Targeting host store-operated Ca(2+) release to attenuate viral infections. Current topics in medicinal chemistry. 2013;13(16):1916-32.

54. Schoeman D, Fielding BC. Coronavirus envelope protein: Current knowledge. Virol J. 2019;16(1):69.

55. Wu YF, Zhao P, Luo X, Xu JC, Xue L, Zhou Q, et al. Chloroquine inhibits Ca(2+) permeable ion channels-mediated Ca(2+) signaling in primary B lymphocytes. Cell Biosci. 2017;7:28-.

56. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive care medicine. 2020;46(4):586-90.

57. Zhu L, Huang L, Zhu Y, Ding X, Zhu G. Calcium signaling involved in bovine herpesvirus 1 replication in MDBK cells. Acta virologica. 2017;61(4):487-91.

58. Zhang L, Liu C, Xiao Y, Chen X. Oxethazaine inhibits hepatitis B virus capsid assembly by blocking the cytosolic calcium-signalling pathway. The Journal of general virology. 2016;97(5):1198-209.

59. Lobeck I, Donnelly B, Dupree P, Mahe MM, McNeal M, Mohanty SK, et al. Rhesus rotavirus VP6 regulates ERK-dependent calcium influx in cholangiocytes. Virology. 2016;499:185-95.

60. Chen JS, Alfajaro MM, Wei J, Chow RD, Filler RB, Eisenbarth SC, et al. Cyclooxygenase-2 is induced by SARS-CoV-2 infection but does not affect viral entry or replication. BioRxiv : The preprint server for biology. 2020;2020.09.24.312769.

61. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardaño JM, Regla-Nava JA, Castaño-Rodriguez C, Fernandez-Delgado R, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology. 2015;485:330-9.

62. Schoeman D, Fielding BC. Coronavirus envelope protein: Current knowledge. Virol J. 2019;16(1):69.

63. Tanaka Y, Sato Y, Sasaki T. Suppression of coronavirus replication by cyclophilin inhibitors. Viruses. 2013;5(5):1250-60.

64. Keyaerts E, Vigen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochemical and biophysical research communications. 2004;323(1):264-8.
65. Yan Y, Zou Z, Sun Y, Li X, Xu KF, Wei Y, et al. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. Cell research. 2013;23(2):300-2.

66. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. [A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-19]. Zhejiang da xue xue bao Yi xue ban = Journal of Zhejiang University Medical sciences. 2020; 49(2):215-9.

67. Magagnoli J, Narendran S, Pereira F, Cummings T, Hardin JW, Sutton SS, et al. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with COVID-19. 2020:2020.04.16.20065920.

68. Wong YK, Yang J, He Y. Caution and clarity required in the use of chloroquine for COVID-19. The Lancet Rheumatology. 2020;2(5):e255.

69. Singh B, Ryan H, Kredo T, Chaplin M, Fletcher T. Chloroquine or hydroxychloroquine for prevention and treatment of COVID-19. The Cochrane database of systematic reviews. 2021;2(2):Cd013587.

70. Duca A, Piva S, Focà E, Latronico N, Rizzi M. Calculated decisions: Brescia-COVID respiratory severity scale (BCRSS)/algorithm. Emergency medicine practice. 2020;22(5 Suppl):Cd1-cd2.

71. Rabie AM. Discovery of taroxaz-104: The first potent antidote of SARS-CoV-2 VOC-202012/01 strain. Journal of Molecular Structure. 2021;1246:131106.

72. Rabie AM. Discovery of (E)-N-[4-(4-cyanobenzylidene)-6-fluoro-3-hydroxyprazaine-2-carboxamide (cyanorona-20): the first potent and specific anti-COVID-19 drug. Chemie zvesti. 2021:1-17.